1-16-89

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

007003

JAN 16 1989

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cyproconazole Technical and Cyproconazole 40 WG: : Evaluation of Toxicity Data Submitted to Support Registration for Nonfood Uses.

TO: Lois Rossi PM-21
Registration Division (TS-767C)

FROM: K. Clark Swentzel K. Clark Swentzel 1/11/89
Acting Section Head
Review Section 2, Toxicology Branch 2 (TS-769C)

THRU: Marcia van Gemert, Ph.D. Marcia van Gemert 1/13/89
Acting Branch Chief
Toxicology Branch 2 (TS-769C)

EPA ID Nos.: 55947-RGG and 55947-RGE
Project Nos.: 8-0814 and 8-0813
Caswell No.: 272E
Registrant: Sandoz Corp.

The registrant has simultaneously submitted applications for the registration of Cyproconazole, for manufacturing use only, and Cyproconazole WG 40, an end-use product turf fungicide product for use on golf courses and sod farms (see attached label and technical information).

Technical product

The appropriate battery of toxicity studies were submitted for the technical product (Table I) based on the application for nonfood uses (158.35), however, the registrant must address several study deficiencies indicated in the attached summary and DERs. The teratogenicity study in rats is not acceptable, however, it is possible to upgrade the core-classification of this study to core-minimum from core-supplementary by submitting requested information. Also, the primary ocular irritation study in rabbits and the in vivo mouse micronucleus test must be repeated.

Some of the studies submitted for the TGAI, which are considered unacceptable, are not required for the requested use. The registrant should refer to Tables I and II in the cover memorandum to determine additional data requirements. The deficiencies indicated in the individual DERs and summary need only be addressed for required studies.

10 of 25

End-use product

The battery of acute studies for the WG 40 formulation (Table II) was also appropriate, however, an acute inhalation study was omitted because the registrant concluded that the product does not contain a significant proportion of respirable particles. Although the registrant submitted particle size data to support this conclusion, a detailed description of the method(s) used as well as examples of findings from tests performed to derive this data should be submitted.

A 21-day dermal toxicity study is required.

Calculation of MOS for developmental toxicity

The skin is a potential source of significant exposure for lawn applicators or those workers/consumers physically handling sod treated with Cyproconazole. While NOELs for developmental toxicity were apparently not attained in either the rat or rabbit study, the LELs (6 and <2 mg/kg/day, respectively) are low and suggest that calculations of MOS may be appropriate--after the issues of NOELs for the studies are resolved and/or new acceptable studies are submitted. Therefore, human exposure data may be required at some time in the future.

007003

Table I

Cyproconazole(TGAI): Toxicity Studies Required under 158.135 for Non-Food Use and Conclusions Regarding the Satisfaction of These Requirements

Test	Required	Satisfied
Acute oral LD_{50}	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	Yes
Primary eye irritation	Yes	No[1/]
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes
21-day dermal	Conditionally	Yes
Teratogenicity-rodent:	Conditionally	No[2/]
Gene mutation	Yes	Yes
Structural aberration	Yes	No[3/]
Other genotoxic effects	Yes	Yes

1/ Data are equivocal, study should be repeated in different animals; core-classification: supplemental.
2/ A NOEL for developmental toxicity could not be determined; additional data is required. Core-classification: supplemental (can be upgraded).
3/ The only study submitted under this category (in vivo mouse micronucleus test) was not acceptable.

007003

Table II

SAN 619F 40 WDG(EP): Toxicity Studies Required under 158.135 for Non-Food Use and Conclusions Regarding the Satisfaction of These Requirements

Test	Required	Satisfied
Acute oral LD_{50}	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes(provisional)	No
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes
21-day dermal	Yes	No

* A description of methodology and examples of findings from tests performed to derive the submitted particle size data must be provided before a decision on the possible requirement of this test can be made.

4

007003

Conclusions from Individual Studies

Technical Grade Active Ingredient

Acute Toxicity Studies

1) Acute Oral LD_{50} Study in the Male and Female Rat with SAN 619F
LD_{50} = 1020 ± 290 mg/kg in males; 1330 ± 346 mg/kg in females. Tox. Category-III
Core classification- minimum

2) SAN 619F: Acute Dermal LD_{50} in the Male and Female Rabbit
LD_{50} > 2000 mg/kg; Tox. Category = III
Core classification- minimum

3) SAN 619F: Acute Dermal Toxicity in the Male and Female Rat
LD_{50} > 2000 mg/kg; Tox. Category = III
Core classification- minimum

4) Four-Hour Acute Dust Aerosol Inhalation Toxicity (LC_{50}) Study with SAN 619F in Rats.
LC_{50} > 5.6 mg/l (includes all airborne particles regardless of size).
Tox. Category = III; Core classification- minimum

5) Irritant Effects on the Rabbit Eye of SAN 619F Technical
Data are equivocal, study should be repeated in different animals.
Core classification- supplementary

6) Irritant Effects on Rabbit Skin of SAN 619F Technical
No primary irritation reaction. Tox. Category = IV. Core classification- minimum

Dermal Sensitization

Skin Sensitization Test in Guinea Pigs

Cyproconazole technical did not induce a sensitization reaction in any of the guinea pigs receiving challenge doses (3ml; up to 5%) in a study using the maximization method of Magnusson and Kligman.

Core-classification: minimum

Subchronic Toxicity Studies

1) Three-Week Dermal Study in Rabbits

Male and female rabbits received repeated dermal applications of Cyproconazole-technical at dosage levels of 50, 250 and 1250 mg/kg for 3 consecutive weeks. There was no macro- or microscopic evidence of induced skin irritation. Evidence of systemic toxicity included inhibited body weight gain and food consumption in high-dose males, increased AST in high-dose males, increased creatinine in high-dose females and increased cholesterol in high-dose males and females. The only noted difference that was statistically significant ($p < 0.05$), in comparison to the concurrent control, was the mean creatinine level in high-dose females. Based on these observations, the LEL was 1250 mg/kg and the NOEL was 250 mg/kg.

Classification: core-minimum

2) Four-Week Study in Rats

Male and female Han Wistar rats were administered Cyproconazole in the diet at levels of 10, 30, 100, 300 and 1000 ppm for 4 weeks. Evidently, this study was performed to determine the dietary levels that should be administered in a subsequent 13-week feeding study in rats. Changes associated with treatment, which were observed in males and females administered the highest dietary level, included decreased body weight and body weight gain, elevated ALT and LDH, increased absolute and relative (% body weight) liver weight and hepatocytomegaly. Elevated BUN and relative testes weights as well as liver vacuolation were observed in high-dose males. Increased cholesterol and relative adrenal weights were noted for high-dose females. The effects present in high mid-dose (300 ppm) males and females were: elevated LDH, increased absolute and relative liver weight and liver vacuolation. Increased relative testes and adrenal weights were seen in high mid-dose males and females, respectively. The LEL and NOEL were 300 ppm (15 mg/kg) and 100 ppm (5mg/kg), respectively.

Core classification: supplementary data (not a Guideline Study)

3) Thirteen-Week Study in Rats

Male and female Han Wistar rats were administered Cyproconazole in the diet at levels of 20, 80 and 320 ppm for 13 weeks; the treatment period was followed by a 4-week recovery period during which additional control and high-dose groups were fed the control diet. Changes associated with treatment, which were observed in rats administered the highest dietary level, include inhibited body weight gain, increased blood levels of creatinine and sodium with a concomitant decrease in calcium, increased liver weights and histological changes in liver. The noted changes in creatinine and calcium were also consistently observed in rats receiving the 20 ppm level but not in those administered 80 ppm. However, since these changes were not seen in treated rats after the recovery period, they should be considered treatment-related effects. A NOEL was not attained, therefore this study is not acceptable for regulatory purposes.

Classification: core-minimum (but NOEL not attained)

4) Thirteen-Week Study in Beagle Dogs

Male and female beagle dogs were administered Cyproconazole in the diet at levels of 20, 100 and 500 ppm for 13 weeks. Changes associated with treatment, observed in both sexes administered the highest dietary level, included "slack muscle tone", inhibited body weight gain, increased platelet counts, decreased: bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein and albumin and increased alkaline phosphatase and gamma glutamyl transferase; decreased food consumption was seen in high-dose males. Increased absolute and relative liver weights and increased relative kidney weights were noted for high-dose males and females; relative brain weights were increased in high-dose females. Histopathologic evidence of liver toxicity in high-dose males and females included hepatocytomegaly, degeneration of single hepatocytes and cytoplasmic inclusions. Evidence of liver toxicity in mid-dose dogs was increased absolute liver weights in males and hepatocytomegaly in males and females.

The LEL in this study, based on adverse effects in liver, was 100 ppm (approximately 4 mg/kg/day and the NOEL was 20 ppm (approximately 0.8 mg/kg/day).

Since this study was not inspected by a QAU during the in-life phase, a data audit, signed and dated by a QA Officer, must be submitted to the Agency before this study can be accepted for regulatory purposes.

007003

Core classification: supplemental (can be upgraded to minimum provided an acceptable QAU audit is provided as noted above)

Developmental Toxicity

1) Teratogenicity Study in Rats

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Wistar/Han rats (25/group) via oral gavage from day 6 through 15 of gestation at dosage levels of 6, 12, 24 and 48 mg/kg. Evidence of maternal toxicity included inhibited body weight gain during treatment at dosage levels of 12 mg/kg and above and decreased body weight and food consumption among females in the 24 and 48 mg/kg dosage groups. However, since the noted differences in maternal body weights were influenced by treatment-related intrauterine effects (e.g., increased number of resorptions, decreased fetal weight etc.), the evidence for maternal toxicity is equivocal.

Evidence of fetal toxicity was apparent from observed dose-related increases in the number of fetuses with supernumerary ribs at dosages of 6 mg/kg (LDT) and above. Embryo/fetal toxicity was apparent at 24 and 48 mg/kg from the following observations: decreased total number of fetuses/dam, decreased number of live fetuses/dam, increased percentage and number of fetal resorptions, decreased body weight and incomplete ossification in phalangeal nuclei and the absence of ossification in calcanea.

There was evidence of teratogenicity in the 24 and 48 mg/kg groups. Hydrocephaly was observed in 1 fetus in the 24 mg/kg and 2 fetuses in the 48 mg/kg groups. Cleft palate was observed in 2 fetuses in the 48 mg/kg group.

The NOEL for developmental toxicity was not determined, based on induced fetotoxicity (supernumerary ribs) at 6 mg/kg. The NOEL for maternal toxicity was 6 mg/kg (equivocal).

The registrant should submit data which show the litter incidence of supernumerary ribs (number of litters/group with the noted change) with appropriate statistical analyses to aid in the determination of a possible NOEL for developmental toxicity in this study.

Core classification - supplemental (can possibly be upgraded to minimum by submitting requested data)

2) Teratogenicity Study in Rabbits

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Chinchilla rabbits (16/group) via oral gavage from day 6 through 18 of gestation at dosage levels of 2, 10 and 50 mg/kg.

Evidence of maternal toxicity, which was not remarkable, included inhibited body weight gain during treatment and decreased food consumption during the initial phase of treatment, both at 50 mg/kg. However, since corrected body weight changes between groups were comparable, the evidence of compound-induced maternal toxicity in this study is not convincing.

Embryo/fetal toxicity, observed at 50 mg/kg, was evident from the decreased number of live fetuses/dam and an increased incidence of non-ossification in certain forelimb and hind limb digits. Evidence of embryo/fetal toxicity at dosages of 10 and 50 mg/kg was indicated by an increased incidence of embryonic and fetal resorptions.

Evidence of teratogenicity included hydrocephalus internus, observed in 1 fetus at

7

each dosage level, and agenesia of the left kidney and ureter in 1 high-dose fetus. Hydrocephaly was also seen at 2 dosage levels in a developmental toxicity study in rats with this test material, however, this anomaly did not occur in the control group of either study.

Since a teratogenic response to the test material was observed at the lowest dose tested, a NOEL for developmental toxicity was not attained in this study. Although evidence of maternal toxicity at 50 mg/kg was not remarkable, the 10 mg/kg dosage level is clearly a no-effect level for maternal toxicity.

This study is not acceptable for regulatory purposes because: 1) a NOEL for developmental toxicity apparently was not attained and 2) the concentrations of test material were not within the acceptable ± 15% of nominal concentration for the mid- and high-dose suspensions immediately after preparation.

Developmental toxicity NOEL: not attained ; <2 mg/kg/day (LDT)
Maternal toxicity NOEL: 10 mg/kg (equivocal)

Core classification: supplementary

Reproductive Toxicity

Two-Generation Study in Rats

Four groups of KFM-Wistar rats were administered technical Cyproconazole at dietary levels of 0(control), 4, 20 and 120 ppm during the pre-mating (10 weeks and 12 weeks, respectively, for the F_0 and F_1 generations), mating, pregnancy and lactation periods to assess the potential reproductive toxicity of the test compound.

Two of the reproductive parameters investigated in parental animals were affected by treatment in F_0 rats only: the duration of gestation at the mid- and high doses was increased and a lower number of implantation sites was seen in high-dose females, both in comparison to respective concurrent control values. Evidence of liver toxicity was seen in high-dose F_0 males (increased lipid storage and relative weight) and females (increased relative weight).

Parameters examined among the offspring which showed treatment-related effects included decreased litter sizes in both the F_1 and F_2 high-dose groups and the F_1 mid-dose group during the early phase of lactation (litters were standardized at day 4 post partum), decreased live birth index in the high-dose F_1 offspring and decreased viability index in the high-dose F_1 and F_2 offspring.

Based on the increased duration of gestation in F_0 dams and the decreased litter sizes observed in F_1 offspring, the LEL in this study was 20 ppm and the NOEL was 4 ppm, which correspond to approximate average dosage levels of 1.7 and 0.4 mg/kg/day, respectively.

Core-classification: minimum (provided test compound stability data and and a description of the sampling technique used for the analyses of dietary levels of test compound are submitted)

Mutagenicity Studies

Gene Mutation

1) Gene Mutation - Ames Salmonella Microsome Reverse Mutation Assay

No evidence of a mutagenic effect at the histidine locus in any of the S. typhimurium strains (TA98, TA100, TA1535, TA1537, TA1538) used at dose levels of 1, 5, 10, 100, 500 or 1000 ug/plate either with or without rat S9 mix.

Acceptable

2) In Vitro HGPRT Gene Mutation Test using Chinese Hamster Ovary Cell Line V79

No indication of mutagenic activity either with or without S9 activation at dose levels of 0, 20, 50, 100 or 200 ug/ml. Test material was soluble only up to 200 ug/ml at which there was little or no evidence of cytotoxicity.

Acceptable

Structural Aberation

Mutagenicity Evaluation of SAN 619F in the In Vivo Mouse Micronucleus Assay

No indication of a mutagenic response (a significantly increased incidence of micronucleated polychromatic erythrocytes) at any of the SAN F dose levels (16.7, 55.7 and 167 mg/kg) for any of the scheduled sacrifice times (24, 48 and 72 hrs).

Not acceptable (additional information regarding the purity of the test material is required).

Other Genotoxic Effects

1) Unscheduled DNA Synthesis in Rat Primary Hepatocytes with SAN 619F

No indication of increased incorporation of 3H-TdR from exposure to SAN 619F either at a single dose level or as part of a dose-related trend. Dose levels: 0.25, 3.3, 6.6, 10 and 25 ug/ml. However, highest dose level should have been greater than 25 ug/ml as there was no indication of a decreased incorporation of 3H-TdR at this level. Also, negative control values were 208.9 dpm, rather than in range of 50-150 dpm.

Not acceptable

2) In Vitro Cell Transformation with Syrian Hamster Embryo (SHE) Cells

No transformation of SHE cells from exposure to SAN 619F for 6 or 48 hrs without S9 activation or as a result of 6-hr exposure to SAN 619F with S9 activation. Dose levels: 20, 50, 100 and 200 ug/ml. No evidence for cytotoxicity at 200 ug/ml but test material precipitated out at concentrations above 200 ug/ml.

Acceptable

007003

3) Unscheduled DNA Synthesis (UDS) Test In vitro in Rat Hepatocytes

No indication of an increased level of incorporation of 3H-TdR in rat hepatocytes exposed to SAN 619 F at 0.15, 0.5, 1.5, 5, or 15 ug/ml with 18-20 hr exposure. Insufficient reporting as to why levels of 50, 100 and 150 ug/ml were "Too toxic to be evaluated for UDS," particularly as LDH activities indicated toxicities below 100% (16%, 61% and 68% respectively).

Not Acceptable (additional information required)

4) Mutagenicity Evaluation of SAN 619F in the Mitotic Non-Disjunction Assay with Saccharomyces Cerevisiae Strain D_6

No increased absolute number of cycloheximide-resistant colonies or of an increased incidence of aneuploids among these colonies following overnight exposure to SAN 619 F at 10, 100, 250, 400, 500 or 550 ug/ml in presence and absence of S9. Range of doses resulted in no, moderate and nearly complete cytotoxicity. No positive control with S9; no information as to how long "overnight" exposure was.

Not Acceptable (S9 activation plus additional information required)

10

10

007003

End Use Product (SAN 619F 40 WDG)

Acute Toxicity Studies

1) Acute Oral Toxicity to Rats of SAN 619F 40 WDG
LD_{50} = 790 mg/kg in males and 1340 mg/kg in females; Tox. category = III
Core-classification: minimum

2) Acute Dermal Toxicity to Rats of SAN 619F 40 WDG
Acute lethal dose > 2,000 mg/kg (only dose tested); Tox. category = III
Core-classification: minimum

3) Irritant Effects on the Rabbit Eye of SAN 619F 40 WDG
No primary irritation reaction; Tox. category = IV
Core-classification: minimum

4) Irritant Effects on the Skin of SAN 619F 40 WDG
Only a slight, transient irritation reaction was observed; Tox. category = IV
Core-classification: minimum

5) Acute Inhalation Toxicity (study not performed) The registrant submitted a letter to the Agency (S. Janousky to L. Rossi, May 19, 1988) which indicated that acute inhalation testing with the 40 WG formulation is not applicable since the bulk of the material consists of large particles that are not inhalable by man. The following data were included to support this contention:

SAN 619F 40 WDG Particle Characterization

Particle size (micrometers)	Percent
1700-2000	1.5
1180-1700	32.2
850-1180	42.6
600-850	15.9
425-600	1.8
< 425	6.4

A description of methodology and examples of findings from tests performed to derive the submitted particle size data must be provided before a decision on the possible requirement of this test can be made. Data which show the proportion of particles in the range inhalable by man (< 10µ) should also be submitted, if they are available.

Dermal Sensitization

Delayed Contact Hypersensitivity in the Guinea-pig with SAN 619F 40 WDG

Repeated topical applications of SAN 619F 40 WDG (50% w/w in distilled water) in guinea pigs did not induce delayed contact hypersensitivity under the conditions of this study.

The procedure used in this study was a modification of the method described in "Delayed Contact Hypersensitivity in the Guinea-pig" Buehler, E.V. (1965), Arch. Dermatol. 91, 171.

11

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 12 through 19 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

X A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

____ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel
Section 3 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section 3 , Tox. Branch (TS-769C)

007003

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity TOX. CHEM. NO.: 272E

MRID NO.: 406077-14

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 6172/84

SPONSOR: Sandoz Corp.

TESTING FACILITY: Sandoz, Basle, Agrotoxicology facility

TITLE OF REPORT: SAN 619F: Acute Dermal Toxicity in the Male and Female Rat

AUTHOR(S): F. Hamburger

REPORT ISSUED: November 15, 1984

CONCLUSIONS: Acute dermal LD_{50} > 2000 mg/kg; Tox. Category = III

Classification: core-minimum

Quality Assurance statement: omitted

007003

Study Title

SAN 619F: Acute Dermal Toxicity in the Male and Female Rat

Test material:

Cyproconazole-technical, lot no. 8404, 95.7% a.i., vehicle-DMSO

Test animals:

Han Wistar rats, 10-16 weeks, 250 ± 50g, acclimation-5 to 8 days

Feed and water:

Feed (KLIBA no. 24-343-7) and water were provided ad libitum.

Environmental parameters:

Light/dark cycle-12 hrs, temperature-23 ± 2°C, relative humidity-30 to 70%, air changes- 15/hr

Procedures

Dosing:

One group of animals consisting of 5 males and 5 females was used in this study. A dose of 2000 mg/kg (4 ml/kg of a 50% solution of test material in DMSO; w/v) was applied to the shaved dorsal skin of an area of 16 cm^2, which represented about 10% of the total surface area. The application area was covered by a porous gauze which was attached by tape at the edges. The animals were placed in Queen Anne teflon collars to prevent oral ingestion of the test material. The gauze was removed and the application sites were rinsed (solution not indicated) at the end of the exposure period.

Observations:

Animals were observed for 1 hr following dosing and at hourly intervals for the remainder of day 1. Observations for symptoms and mortality were performed twice daily for the remainder of the 14-day observation period. If appropriate, the following data were recorded: 1) approximate time of death, 2) the nature, severity, approximate time of onset and duration of each symptom and 3) individual body weights on day 1, 7 and 14.

Postmortem examination:

All animals were sacrificed at the termination of the observation period and examined macroscopically.

Results

Treatment appeared to inhibit bodyweight gain in both males and females, however, without concurrent control animals, it is difficult to provide a conclusive evaluation of the data.

	Mean bodyweights(g)		Bodyweight change(%) (Rel. to Day 1)	
Day	Males	Females	Males	Females
1	271	254	---	---
7	275	241	+1.5	-5.1
14	307	249	+11.6	-2.0

Evidently bodyweight gain was inhibited in males during the first week of the observation period while females actually lost bodyweight during the first week. However, it should be noted that the females had reached the slow phase of the maturation growth curve, which might explain why the mean bodyweight had not reached the day 1 value by the end of the observation period.

No treatment-related symptoms other than weakness (observed for 2-48 hrs in males and 2-24 hrs in females) were reported.

No mortalities occurred during the study, therefore, the acute dermal LD_{50} in rats was not determined. Since 2000 mg/kg did not induce toxicity, the administration of additional dosage levels is not necessary (Subdivision F Guidelines).

Conclusion: Acute dermal LD_{50} > 2000 mg/kg; Tox. Category = III

Core classification: minimum

007003

Reviewed by: K. Clark Swentzel
Section 3 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section 3 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation Toxicity TOX. CHEM. NO.: 272E

MRID NO.: 406077-15

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 052975

SPONSOR: Sandoz Corp.

TESTING FACILITY: Research & Consulting Company AG

TITLE OF REPORT: Four-Hour Acute Dust Aerosol Inhalation Toxicity (LC_{50}) Study with SAN 619F in Rats

AUTHOR(S): L. Ullmann

REPORT ISSUED: September 19, 1985

CONCLUSIONS: LC_{50} of tested material > 5.6 mg/l; Tox. Category = III

Classification: core-minimum

Quality Assurance statement: signed and dated

Study Title

Four-Hour Acute Dust Aerosol Inhalation Toxicity (LC_{50}) Study with SAN 619F in Rats.

Test material:

Cyproconazole, powder, 95 ± 1 %, batch no. 8507

Test animals:

KFM-Han Wistar rats, 10 weeks old, males- 247 to 284g, females- 203 to 227g, 5 males and 5 females/group, acclimated at least 1 week.

Feed and water:

Pelleted standard Kliba 343, Batch 27/85 rat maintenance diet, and tap water were provided ad libitum.

Environmental parameters:

During the 15-day observation period: room temperature- 22 ± 2°C, relative humidity- 55 ± 10%, 12 hr light/dark cycle, 10-15 air changes/hr.

Procedures

Exposure:

Five rats/sex/group received a single 4-hour exposure (nose only) to airborne dust particles of test material. The mean exposure levels were 2606 (low dose) and 5645 (high dose) mg/m^3. The nominal concentrations for these analyzed airborne levels were 62 and 279 g/m^3, respectively.

Chamber parameters:

Exposures were in a 100 liter polyvinyl-chloride dynamic chamber in which the air flow was 1000l/hr and the air pressure was 3 atmospheres. The chamber environmental conditions, which were monitored 8 times during the exposure period, were: temperature- 23°C, relative humidity- 40% and oxygen content- 20%.

Determination of exposure concentration:

Determined 5 times gravimetrically on selectron filters (pore size- 0.2 um, 50 mm in diameter).

Particle size determination:

Gravimetric determination was performed 3 times using an 8-stage particle sizing sampler with selectron filters (pore size- 0.2 um, 76 mm in diameter).

Post treatment observation period:

The following observations were made during the 15-day observation period:

Symptoms and mortality: 4 times during the first day and daily thereafter.

Body weights: At days 1 (day of exposure), 8 and 15 of the study.

Macroscopic and microscopic pathology:

Sections of nasal cavity, lungs with mainstem bronchi, liver, kidneys, adrenal glands and all gross lesions from all rats in group 2 (high dose) were examined microscopically. Only gross lesions in group 1 rats (low dose) were examined microscopically.

Results

Symptoms and mortalities:

The investigator indicated that slight sedation, dyspnea and ruffled fur was uniformly observed in both sexes in each group, however, these symptoms were not evident within 24 hrs after exposure. Treatment did not induce any mortalities in either group.

Body weights:

Treatment had no apparent effect on bodyweight gain during the observation period.

Macroscopic and microscopic findings

The only macroscopic change noted was in the kidneys of 2 low dose males in which a dark red color was observed at the corticomedullary junction. The only microscopic changes noted were mineralization in kidneys at the corticomedullary junction in 4 high dose females and blood accumulation in the bronchi and alveloli of one high dose male and 2 high dose females. None of these changes were clearly related to treatment.

Chamber parameters:

Exposure concentrations (mg/m^3)

	Range	Mean	Stand. Dev.
Low dose:	2492-2710	2602	± 93
High dose:	5490-5802	5645	± 142

Oxygen content:

The O_2 content was consistently 20% for both groups.

Relative humidity (%)

Low dose: 40

High dose: 48-54

Temperature(°C):

Low dose: 22-23

High dose: 22-24

Particle sizes(approximate proportion)

	2 um or less	5 um or less
Low dose:	4%	42%
High dose:	22%	79%

LC_{50} determination:

Since the test material did not induce any mortalities at either exposure level, the LC_{50} was not attained in this study.

Only 4% of the airborne particles at the low dose level were 2 um or smaller, therefore, the animals did not inhale a significant amount of test material (4% of 2606 mg/m^3 = 104 mg/m^3). However, even though only 22% of the airborne particles at the high dose level were 2um or smaller, the total airborne concentration was high, therefore, the airborne concentration of smaller particles was relatively high (22% of 5645 mg/m^3 = 1242 mg/m^3). At this concentration, inhalation of 105 ml/min would result in respiratory exposure to approximately 31 mg of test material over a 4 hr period (approximately 190 and 130 mg/kg for males and females, respectively). Therefore, it is TB's opinion that the high dosage level was adequate.

Conclusion: LC_{50} of tested material > 5.6 mg/l; Tox. Category = III

Core classification: minimum

007003

Reviewed by: K. Clark Swentzel
Section 3 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section 3 , Tox. Branch (TS-769C) M. van Gemert 7/28/88

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation TOX. CHEM. NO.: 272E

MRID NO.: 406077-16

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 8817D/SNC 17/SE

SPONSOR: Sandoz Corp.

TESTING FACILITY: Huntingdon Research Centre, Ltd.

TITLE OF REPORT: Irritant Effects on the Rabbit Eye of SAN 619F Technical

AUTHOR(S): M. Liggett

REPORT ISSUED: December 14, 1987

CONCLUSIONS: The irritation data generated in this study are equivocal, therefore, this study should be repeated in 6 different rabbits.

Classification: core-minimum

Quality Assurance statement: signed and dated

007003

Study Title

Irritant Effects on the Rabbit Eye of SAN 619F Technical

Test material:

Cyproconazole-technical, batch no. 8507, purity of material not provided

Test animals:

New Zealand white male rabbits, 2.9 to 3.5 kg, 12 to 15 weeks of age, acclimation period not given

Feed and water:

SDS Standard Rabbit Diet and tap water provided ad libitum

Environmental parameters:

Room temperature- 19°C, relative humidity- 30 to 70%, 19 air changes/hr, 12 hr light/dark cycle

Procedures

Treatment and grading:

The eyes of each animal were examined prior to instillation of the test substance

in order to detect possible corneal damage or conjunctival inflammation. A 60 mg amount of SAN 619F technical, the weight occupying a volume of 0.1 ml, was placed into the lower everted lid of one eye of each 6 animals; the eyelids were then held together for 1 second. The remaining eye served as the untreated control.

The eyes were examined at 1 hr and 1, 2, 3, 4 and 7 days post treatment. Grading and scoring of the ocular lesions were performed using the numerical scoring system indicated on appended pages 1 and 2.

Systemic toxicity: All animals were observed daily.

Results

The numerical scores for the ocular reactions observed in this study are shown on appended page 3. A positive irritation reaction was observed in two rabbits. A diffuse red coloration of the conjunctivae was observed in one animal at 1 and 24 hrs post treatment. This reaction was not evident for the remainder of the observation period. Obvious swelling with partial eversion of the lids was seen in the second animal at 1 hr post treatment only. Discharge, moistening the lids and hairs adjacent to the lids, was observed in 4 of the rabbits at the 1 hr interval only. No corneal damage or iridial inflammation was observed. No irritation reactions were observed 2 or 3 days after instillation of the test substance. The investigator did not see any signs of systemic toxicity in any of the test animals.

28

Since a test substance is considered an ocular irritant if a positive irritation reaction is observed in 4/6 rabbits and a non-irritant if a positive reaction is seen in 1/6 rabbits, it is TB's opinion that the data generated in the present study do not provide a basis for a conclusion regarding the ocular irritation potential of the subject compound.

Conclusion

The irritation data generated in this study are equivocal, therefore, this study should be repeated in 6 different rabbits.

Core classification: supplemental (equivocal data; purity of the test material was not provided)

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 30 through 32 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007003

Reviewed by: K. Clark Swentzel
Section 3 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section 3 , Tox. Branch (TS-769C) 7/25/88

DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irritation TOX. CHEM. NO.: 272E

MRID NO.: 406077-17

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 871672D/SNC 16/SE

SPONSOR: Sandoz Corp.

TESTING FACILITY: Huntingdon Research Centre, Ltd.

TITLE OF REPORT: Irritant Effects on Rabbit Skin of SAN 619F Technical

AUTHOR(S): M. Liggett

REPORT ISSUED: November 27, 1987

CONCLUSIONS: The test material did not induce primary dermal irritation; Tox. Category = IV

Classification: core-minimum

Quality Assurance statement: signed and dated

33

007003

Study Title

Irritant Effects on Rabbit Skin of SAN 619F Technical

Test material:

Cyproconazole-technical, batch no. 8507, purity of material not provided

Test animals:

New Zealand white male rabbits, 2.3 to 3.3 kg, 10 to 14 weeks of age

Feed and water:

SDS Standard Rabbit Diet and tap water provided ad libitum

Environmental parameters:

Room temperature- 19°C, relative humidity- 30 to 70%, 19 air exchanges/hr, 12 hr light/dark cycle

Procedures

Treatment:

Hair was removed from the dorso-lumbar region of each rabbit approximately 24 hrs before application of the test material to expose a 10 cm^2 area of skin. A 0.5 g dose of Cyproconazole was applied under a 2.5 cm^2 gauze pad moistened with 0.5 ml distilled water to one intact skin site on each animal. Each treatment site was occluded with elastic adhesive dressing for a 4-hr period. The animals were placed in their cages unrestrained during the exposure period. At the end of the exposure period, the semi-occlusive dressing and gauze pad were removed and the treatment site was washed using water to remove any residual rest material.

Observations and grading:

Examination of the treated skin was made on Day 1 (approximately 30 minutes after removal of the patches) and on Days 2, 3, and 4. Dermal reactions were graded and scored according to the numerical scoring system shown on appended page 1.

All animals were observed daily for signs of systemic toxicity.

Results

The numerical scores on appended page 2 show that an irritation reaction was not observed on any animal at any interval.

There were no signs of systemic toxicity in any animal.

34

007083

00

Conclusion

The administration of Cyproconazole-technical to intact skin sites in rabbits d: induce a primary irritation reaction in this study. Tox. Category = IV

Core classification- minimum

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 36 through 37 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel 7/13/88 007003
Section 3 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. M. van Gemert 7/25/88
Section 3 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization TOX. CHEM. NO.: 272E

MRID NO.: 406243-04

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 6390/85

SPONSOR: Sandoz Corp.

TESTING FACILITY: Sandoz Agrotoxicology, Basle

TITLE OF REPORT: SAN 619F: Skin Sensitization Test in Guinea Pigs

AUTHOR(S): F. Hamburger

REPORT ISSUED: July 25, 1985

CONCLUSIONS: Cyproconazole technical did not induce a sensitization reaction in any of the guinea pigs (19) receiving challenge doses (3ml; up to 5%) in a study using the maximization method of Magnusson and Kligman.

Classification: core-minimum

Quality Assurance statement: signed and dated

007003

Study Title

SAN 619F: Skin Sensitization Test in Guinea Pigs

Test material

Cyproconazole, technical: Lot No. 8405, 94.4% a. i.;
Vehicle - DMSO
Positive control - DNCB (1-chloro-2,4-dinitrobenzene)

Test animals

Albino guinea pig (strain and sex not specified), 4 weeks at delivery (age at test initiation not given), body weight ranges: pretest - 245 to 260g, main test - 180 to 280g; acclimation = 8 days.

Feed and water

Diet: KLIBA no. 24-342; water was provided ad libitum.

Environmental parameters

Temperature: 23 ± 2° C, 12-hr light/dark cycle, rel. humidity: 30-70%, 15 air changes/hr

Procedures

Animal groups

Two animals were assigned to each group (2) in the pretest and 20 were distributed to each group (3) in the main test. Group assignments were based on random numbers.

Treatment (appended page 1)

All administrations were preceded (24 hr) by hair clipping at the dorsal application sites.

Pretest

Pretest animals were administered 3ml of either a 1% or 5% solution of SAN 619F in DMSO or Bacto complete adjuvant on intact skin at days 8 and 22 to evaluate possible primary dermal irritation. Each treatment site was covered with a patch which was affixed with impermeable plastic tape.

Main test

Induction period (Day 1; intradermal administration)

Negative control: adjuvant alone (2 sites), DMSO alone

Test group: adjuvant alone, 5% SAN 619F in DMSO, 2% SAN 619F in adjuvant (5% not soluble)

Positive control: adjuvant alone, 0.1% DNCB in ethanol, 0.1% DNCB in adjuvant

007003

Optimization (Day 8 to 10; epicutaneous, patch 48 hrs)

Negative control: DMSO only

Test group: 5% SAN 619F in DMSO

Positive control: 1% DNCB in ethanol

Following hair clipping on Day 7, the application sites were treated with aqueous 10% sodium lauryl sulfate.

Challenge (Day 22 to 23, epicutaneous, patch 24 hrs)

Negative control: 5% SAN 619F in DMSO

Test group: 5% SAN 619F in DMSO

Positive control: 1% DNCB in ethanol

Observations

Body weights

Weights were recorded on Days 1, 7, 15, 22 and 25 (termination).

Evaluation of skin reactions

Evaluations were performed 24 hrs after patch removal following optimization treatments and at 24, 48 and 72 hrs after patch removal following challenge treatments according to the scheme of Magnusson and Kligman as shown on appended page 2. The grading system is based primarily on the proportion of animals that react with various grades of redness after challenge treatments.

Results

Pretest skin evaluation

No skin reactions were observed from either 1% or 5% SAN 619F in DMSO or adjuvant following the Day 8 and Day 22 applications. The 5% level was not soluble in adjuvant so the investigator decided to use 2% in the main test.

40

Main test skin evaluation

Skin reactions were not induced in the negative control or test group (appended pages 3 and 4, respectively) after either the maximization or challenge applications. The challenge administration of positive control caused a positive reaction in every animal (appended page 5) which tended to increase in intensity during the 3 day observation period. The positive control group was not tested concomitantly with the other groups (approximately 7 weeks after the main study). The investigator did not provide an explanation for this schedule.

Body weights

Even though mean terminal body weights were comparable between groups (329.0, 331.3 and 339.8 for negative control, test group and positive controls, respectively), mean body weight gains were 91.0 and 77.1 % (relative to negative controls) for the test and positive control groups, respectively. However, SAN 619F did not have an apparent effect on body weight gain in the main test.

Mortality

One animal died in the test group on day 11. The investigator indicated that the cause of death was pneumonia.

Conclusion

Cyproconazole technical did not induce a sensitization reaction in any of the 19 guinea pigs receiving challenge doses (3ml; up to 5%) in a study using the maximization method of Magnusson and Kligman. The positive control (1% DNCB) caused sensitization reactions in all treated animals (20) in a subsequent test using the same method.

Core classification: minumum

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 42 through 46 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel K. Clark Swentzel 8/5/88
Section 3 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. M van Gemert 8/5/88
Section 3 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Three-week Dermal TOX. CHEM. NO.: 272E

MRID NO.: 406243-04

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): LMP415-RB

SPONSOR: Sandoz Corp.

TESTING FACILITY: Sandoz Agrotoxicology

TITLE OF REPORT: SAN 619F: 3-week Dermal Study in Rabbits

AUTHOR(S): S. Carpy

REPORT ISSUED: April 20, 1988

CONCLUSIONS: Male and female rabbits received repeated dermal applications of Cyproconazole-technical at dosage levels of 50, 250 and 1250 mg/kg for 3 consecutive weeks. There was no macro- or microscopic evidence of induced skin irritation. Evidence of systemic toxicity included inhibited body weight gain and food consumption in high-dose males, increased AST in high-dose males, increased creatinine in high-dose females and increased cholesterol in high-dose males and females. The only noted difference that was statistically significant ($p < 0.05$), in comparison to the concurrent control, was the mean creatinine level in high-dose females. Based on these observations, the LEL was 1250 mg/kg and the NOEL was 250 mg/kg.

Classification: core-minimum

Quality assurance statement: signed and dated

Study Title

SAN 619F: 3-Week Dermal Study in Rabbits

Test Material

Cyproconazole-technical, 95.6%, Lot no. 8507, described as brownish powder, vehicle-partially demineralized water.

Dosage levels: 50, 250 or 1250 mg/kg
Controls: appropriate volume of demineralized water

Test Animals

New Zealand white rabbits:

Status at the initiation of the test:

Males- approximately 14 weeks of age; 2.58 ± 0.140 kg
Females- approximately 17 weeks of age; 2.61 ± 0.045 kg

Acclimation: approximately 2 weeks

Identification: individual ear marking and colored cage cards with animal no.

Number/sex/group: 5

Housing: individually , in steel cages

Food and Water

Diet: Kliba, pelleted diet No. 23-341-1
Water: municipal water ad libitum

Environmental Parameters:

Temperature: 21 ± 2°C; relative humidity: 50 ± 20%; light/dark cycle: 6-18 hrs each

Procedures

Test material administration

Approximately 10% of the dorsal surface was clipped at least twice during each treatment week to remove hair from the application sites.

An aqueous paste was prepared immediately before each application period by mixing 2 ml H_2O with each g of powdered substance. The paste was spread evenly over the treated area by means of a flexible plastic spatula. Dosages were determined gravimetrically by weighing each container after application. Treated areas were covered with two layers of gauze beneath a porous plastic screen (mesh 2 mm i.d.) attached by means of 20 cm wide elastic cloth. The duration of treatment was 6 hours/day, 5 days/week for the first 2 weeks and 7 days during the third week.

48

007083

Observations

Symptoms and skin reactions

Prior to treatment and 6 hours after treatment each day, all animals were examined for symptoms of toxicity. Skin irritation was graded daily by the method of Draize (appended page 1) prior to application and half an hour after patch removal.

Body weight

Body weights were recorded twice weekly during treatment and at termination.

Food consumption

Food consumption was determined gravimetrically twice each week.

Clinical Determinations

Hematology and clinical chemistry parameters were determined on all animals 3 and 4 days before initiation of treatment and at termination (day 19). The animals were fasted 24 hours before collection of blood from the marginal ear vein. The following parameters were investigated:

Hematology

- Hematocrit
- Hemoglobin
- Erythrocytes
- Mean cell volume
- Mean corpuscular hemoglobin
- Mean corpuscular hemoglobin concentration
- Leukocytes (total and differential)
- Platelet counts

Clinical chemistry

- Hemolysis
- Glucose
- Blood urea
- Creatinine
- Total bilirubin
- Albumin
- Total protein
- Electrolytes
- Total cholesterol
- Triglycerides
- Alanine aminotransferase
- Aspartate aminotransferase
- Alkaline phosphatase
- Gamma glutamyl transpeptidase
- Globulins

Gross Examination

All surviving animals were subjected to gross external and internal examination. The following organs were extracted and weighed: liver, kidneys, adrenals and testes.

Microscopic Examination

The following organs and tissues from all animals were preserved in 4% formaldehyde

49

for subsequent histological processing: treated and untreated clipped skin, liver, kidneys, testes ovaries, adrenals and any tissue showing lesions or other alterations.

Statistical Analysis

Body weight, food consumption, hematology, clinical chemistry, organ weight and organ weight/bodyweight ratios were analysed statistically for differences between treated and control values (F-test: if the variances were homogeneous, the t-test was performed; if variances were not homogeneous, the U-test of Mann-Whitney was performed). Hematology and clinical chemistry data were also analysed for differences between termination and pre-exposure times.

Results

Actual Dosages Administered

The investigator calculated the following mean dosages (combined sexes) for the low, medium and high dosage groups, respectively:

mg/kg
L- 49.76
M- 249.05
H- 1247.20

Mortalities

None of the animals died or became moribund during the study.

Skin Reactions

No treatment-related skin irritation reactions were observed. Sporadic mild erythema was noted, however, this reaction was neither treatment- nor dose- related.

Body weight

Body weight gain was inhibited in high-dose males only. None of the differences between mean weights for treated groups and respective control groups at termination were statistically significant.

Intergroup and Chronological Comparisons of Mean Body Weights

Males	Wt.(kg) (Day 1)	vs. cont.(%)	Wt.(kg) (Day 22)	vs. cont.(%)	% Gain (Day 1-22)
Control-	2.676	----	3.044	----	13.8
Low dose-	2.562	-4.26	2.880	-5.39	12.4
Med. dose-	2.528	-5.53	2.892	-4.99	14.4
High dose-	2.556	-4.48	2.768	-9.07	8.3
Females					
Control-	2.584	----	2.852	----	10.4
Low dose-	2.574	-0.39	2.938	+3.02	14.1
Med. dose-	2.868	+10.99	2.934	+2.88	2.3
High dose-	2.598	+0.54	2.927	+2.63	12.7

50

The most significant period for inhibition of body weight gain in high-dose males was Days 1-4 when 4/5 animals lost weight resulting in a mean change of -0.114 kg. This group gained weight at all other weighing intervals during the study.

Food Consumption

Intergroup Comparisons of Mean Food Consumption Values (Days 1-21)

g/day(sd)

	Males	Females
Control-	193.2(4.67)	179.2(20.8)
Low dose-	175.5(16.8)	180.0(17.6)
Med. dose-	189.2(15.9)	178.8(9.1)
High dose-	167.7(35.6)	181.3(19.9)

Consistent with the above body weight data, food consumption was decreased in high-dose males. The lowest consumption in this group occurred on days 1-4 (107 ±67.5g).

Clinical Investigations

The only noteworthy variation was observed in the platelet count for low- and mid-dose males:

$10^3/mm^3$(sd)

Control	LD	MD	HD
492.2(73.3)	374.8(73.8)	327.6(100.5) p< 0.01	546.6(35.8)

The low- and mid- dose male groups each had 1 animal with a low count (297 and 178 $10^3/mm^3$, respectively). Also, in the absence of a dose-response relationship, these data do not appear to toxicologically significant.

None of the other hematological parameters revealed an adverse effect from the test material.

Clinical chemistry

Increased mean values were observed at termination for the following parameters:

		Control	LD	MD	HD
AST in males					
U/L(sd)		15.44(3.94)	13.94(3.77) ns	17.01(9.59) ns	19.34(7.61) ns
Creatinine in females					
uMol/L(sd)		102.22(7.32)	122.48(17.80) ns	99.12(18.70) ns	128.80(14.31) p< 0.05
Cholesterol in males and females					
MMol/L(sd)	M-	1.262(0.275)	1.190(0.319) ns	1.284(.247) ns	1.428(.320) ns
	F-	1.356(.278)	1.430(.382) ns	1.456(.405) ns	1.648(.349) ns

58

51

AST values increased in mid- and high-dose males; neither increase was statistically significant. Each group had 1 animal with an elevated value. Slight dose-related increases in AST were also observed in treated females, however, these increases were not statistically significant because the mean control value was elevated by high levels in 2 animals (22.3 and 140.0 U/L).

	Control	LD	MD	HD
AST in females U/L	39.31(56.53)	11.66(1.60)	13.10(4.85)	16.82(4.88)

Based on dose-response relationships, the noted increased levels of creatinine in HD females, cholesterol in HD males and females and AST in HD males and females appear to be treatment-related.

Bilirubin levels increased in mid- and high-dose males and decreased in mid- and high-dose females; none of the changes were statistically significant, nor did they appear to be biologically significant.

No other noteworthy changes in clinical chemistry parameters were observed.

Organ Weights

None of the differences in organ weights between control and treated groups were statistically significant; slight increases in relative and absolute liver weights in high-dose males and females and relative adrenal weights in high-dose males were measured.

Rel. liver wts	% body wt(sd)	
	Males	Females
Control-	2.923(0.395)	2.647(0.249)
LD-	2.855(0.179)	3.151(0.175)
MD-	3.164(0.305)	2.834(0.430)
HD-	3.435(0.307)	3.236(0.282)

Ab. liver wts	g (sd)	
Control-	89.52(17.8)	75.5(8.8)
LD-	82.24(8.0)	92.4(2.5)
MD-	92.0(15.8)	83.4(16.2)
HD-	95.02(12.9)	94.7(9.0)

Rel. adrenal wts	% body wt(sd)	
Control-	0.0078(0.0011)	0.0080(0.0019)
LD-	0.0088(0.0021)	0.0079(0.0012)
MD-	0.0076(0.0008)	0.0083(0.0012)
HD-	0.0091(0.0015)	0.0087(0.0009)

In the absence of histologic changes in these organs, the noted organ weight changes can not be conclusively associated with treatment.

52

007013

Macroscopic changes

There was no evidence that sporadic changes observed in spleen (enlargement), liver (lobular discoloration) and kidney (irregular surface) were associated with the administration of test material.

Microscopic changes

The investigator did not find histologic evidence of skin irritation at the application sites. Interstitial nephritis and mononuclear hepatitis were observed with comparable frequency among all groups. These changes, as well as others observed sporadically, did not provide histological evidence of an adverse effect from treatment.

Conclusion

The following changes relative to controls were noted after repeated dermal applications of Cyproconazole in rabbits for 3 weeks at dosage levels of 50, 250 and 1250 mg/kg: inhibited body weight gain and food consumption in HD males, increased AST in HD males, increased creatinine in HD females and increased cholesterol in HD males and females. The noted increased creatinine value was the only one of these changes that was statistically significant ($p < 0.05$) in comparison to the mean value for concurrent controls. AST was also increased in HD females relative to other treated groups but not to the concurrent controls which had a relatively high mean level due to elevated levels in 2 animals.

Relative and absolute liver weights in HD males and females as well as relative adrenal weights in HD males were elevated, however, none of these differences were statistically significant in comparison to respective mean concurrent control values. Also, it is unlikely that any of these differences were biologicallly significant since treatment-related histological changes were not observed in these organs.

Based on the noted treatment-related effects on body weight gain, food consumption and clinical chemistry values, the LEL for Cyproconazole in this study is 1250 mg/kg and the NOEL is 250 mg/kg.

Core-classification: minimum

Reviewed by: K. Clark Swentzel K. Clark Swentzel 9/15/88
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. Marcia van Gemert 9/30/88
Section 2 , Tox. Branch (TS-769C)

007003

DATA EVALUATION REPORT

STUDY TYPE: 13-Week Feeding Study TOX. CHEM. NO.: 272E

MRID NO.: 406243-04

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 353/354 R

SPONSOR: Sandoz Corp.

TESTING FACILITY: Sandoz Agrotoxicology

TITLE OF REPORT: SAN 619F: 13-Week Feeding Study in Rats

AUTHOR(S): C. Skinner

REPORT ISSUED: April 1986

CONCLUSIONS: Male and female Han Wistar rats were administered Cyproconazole in the diet at levels of 20, 80 and 320 ppm for 13 weeks; the treatment period was followed by a 4-week recovery period during which additional control and high-dose groups were fed the control diet. Changes associated with treatment, which were observed in rats administered the highest dietary level, include inhibited body weight gain, increased blood levels of creatinine and sodium with a concomitant decrease in calcium, increased liver weights and histological changes in liver. The noted changes in creatinine and calcium were also consistently observed in rats receiving the 20 ppm level but not in those administered 80 ppm. However, since these changes were not seen in treated rats after the recovery period, they should be considered treatment-related effects. A NOEL was not attained, therefore this study is not acceptable for regulatory purposes.

Classification: core-minimum (but NOEL not attained)

Quality assurance statement: Signed and dated

54

TEST MATERIAL

Concentration of technical material = 95.7%; Lot no.: 8405; Code name: SAN 619 F; Isomer composition: 619 A = 54.3%, 619 B = 41.4%; Description: white powder.

TEST ANIMAL DATA

Rats--Han Wistar; Age at initiation of study = 7 weeks; Body weights at study initiation: males = 176 ± 2.6g; females = 149 ± 1.3g; Identification: cage cards and individual ear marks.

Group assignment: animals were randomly selected and 15 rats/sex were assigned to 1 control and 3 dosage groups for the 90-day dosage period. Also, 15 rats/sex were assigned to an additional control or high-dose group for a 4-week recovery study.

Acclimation: 1 week

Housing: Individually in Macrolon® cages (size 3) with wood chips

Food: Kliba, powdered diet no. 21-343-4: ad libitum

Water: municipal tap water in polypropylene bottles: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 23 ± 2°C; Relative humidity - 50 ± 20%.

METHODS

Administration of test material

The test material was mixed with the diet from a 1% premix at levels of 20, 80 or 320 ppm which was fed to the test animals for 13 weeks; the recovery groups (1 control and 1 high-dose) were maintained on untreated diet for 4 weeks following the treatment period. Test diet mixtures were prepared weekly.

Test Diet Analysis

Cyproconazole levels in the premix and final diets were analysed prior to study initiation and at monthly intervals during the study by the registrant.

Results: The mean concentrations of Cyproconazole (4 analyses) were:

Dose(ppm)	Mean concentration(ppm)	Percent of nominal concentration
20	20.1	100.6
80	67.8	84.7
320	287.5	89.8

Symptoms and Mortality

The investigator indicated that examinations included daily inspection of skin,

007003

fur, feces, urine, eyes, ocular mucous membranes, respiration, circulatory and neurological activity.

Results: There were no mortalities in the study. The only clinical symptom noted in dosed animals by the investigator was piloerection, which was observed in males only (8/15 mid-dose and 14/30 high-dose).

Body Weight

Measured on day 1 and weekly thereafter.

Results: Body weight gain was slightly inhibited in high-dose males and mid- and high-dose females, however, the body weight deficits between high-dose males and females and the respective concurrent control values at the end of the treatment period were only -4.5 and -6.0%, respectively.

Body weight gain(g): 0-13 weeks

Dose(ppm)	0	20	80	320
Males	142.5	139.9	138.5	131.6
Rel.to Cont.	---	-1.8%	-2.8%	-7.6%
Females	66.0	67.6	61.1	59.2
Rel.to Cont.	---	+2.4%	-7.4%	-10.3%

Food Consumption

Determined weekly. Wasted food was salvaged and weighed.

Results: Only slight decreases in food consumption were noted for high-dose males and mid- and high-dose females.

Compound Consumption

Dietary intakes (mg/kg/day) were calculated by the investigator from body weight, food consumption and nominal dietary concentrations of test material.

Results: Adjustments to the investigator's calculated compound intake values, based on analytical data rather than nominal compound levels, gave the following mean dosage levels.

Mean intake(mg/kg/day)

Nominal dose (ppm)	20	80	320
Males	1.5	5.4	21.4
Females	1.9	5.9	27.9

Laboratory Investigations

Hematology and blood chemistry parameters were investigated in 10 males and 10 females per group at weeks 4, 8 and 13 during the treatment period and at weeks 14 and 18 during the recovery period. Animals were selected by randomized numbers generated by a computer. The rats were fasted for 16-18 hours prior to collection of blood which was drawn from the sublingual vein.

Hematology parameters

Hematocrit-Hct
Hemoglobin-Hgb
Erythrocytes-RBC
Mean cell volume-MCV
Mean corpuscular hemoglobin-MCH
Mean corpuscular hemoglobin concentration-MCHC
Leukocytes (total and differential)-WBC
Platelets
Reticulocytes

Results: A statistically significant decrease in the hematocrit with a concomitant increase in the MCHC were observed in mid- and high-dose males at weeks 4 and 8. These changes do not appear to be biologically significant since they were observed in one sex at 4 and 8 weeks only. Other changes observed during the treatment and recovery periods were sporadic and did not appear to be related to dosing.

Hematocrit and Mean Corpuscular Hemoglobin Concentration in Male Rats

Dose(ppm)	Hct(%)			MCHC(%)		
Weeks--	4	8	13	4	8	13
	Mean/S.D.					
0	48.52	50.08	48.91	35.64	35.34	36.00
	1.51	1.17	1.16	0.67	0.69	0.56
20	48.68	51.54	46.82*	35.58	34.59	36.08
	1.92	3.66	1.74	0.34	2.25	0.35
80	46.48*	45.70**	48.00	36.51**	37.65**	36.15
	1.53	1.90	1.49	0.27	0.62	0.49
320	45.84**	46.09**	48.25	36.68**	37.50**	36.35
	1.42	2.12	1.03	0.51	1.36	0.49

*p<0.05; **p<0.01 (Dunnett's t test)

Clinical chemistry parameters

Hemolytic score
Glucose
Urea
Bilirubin
Albumin
Total protein
Sodium
Potassium
Calcium
Chloride
Total cholesterol
Glutamic pyruvic transaminase(SGPT)-ALT
Glutamic oxaloacetic transaminase(SGOT)-AST
Alkaline phosphatase
Creatinine

Results: Apparent treatment-associated changes in sodium, creatinine and calcium

levels were observed. Sodium levels were decreased slightly in mid- and high-dose males ($p<0.05$) at week 4 but were elevated in all dosed males ($p<0.01$) and high-dose females ($p<0.05$) at week 13. Creatinine was increased in low- and high-dose females ($p<0.01$) at all intervals during the treatment period and in low and high-dose males at weeks 8 ($p<0.01$) and 13 ($p<0.01$ and 0.05 for low- and highdose, respectively). Calcium levels were depressed in low- and high-dose females at week 4 ($p<0.05$), low-dose males ($p<0.01$) and females ($p<0.05$) at week 8 and in low- ($p<0.01$) and high-dose ($p<0.05$) males and mid- ($p<0.05$) and high-dose ($p<0.01$) females at week 13.

The noted changes in creatinine and calcium occurred consistently in low and high-dose animals of both sexes, but rarely in mid-dose animals. The investigator did not offer a possible explanation for this observation.

During the recovery period, elevated levels of sodium in males and females ($p<0.01$) and creatinine in males ($P<0.05$) were observed at week 14 but not at week 18.

Changes in Sodium, Creatinine and Calcium levels

Sodium (MMol/L) Mean/S.D.

Males	Treatment Period			Recovery Period	
Weeks--	4	8	13	14	18
Dose (ppm)					
0	140.2	144.4	144.9	143.4	145.5
	1.9	1.2	1.7	1.3	1.0
20	141.0	145.0	147.4**	--	--
	1.2	1.2	1.1		
80	138.4*	145.2	147.6**	--	--
	1.0	1.1	1.1		
320	138.7*	145.6	147.0**	149.2**	145.9
	0.9	1.8	1.4	1.5	1.1
Females					
0	145.1	143.9	142.1	143.7	145.1
	1.6	2.0	0.7	0.8	1.9
20	146.3	143.9	142.4	--	--
	2.1	1.8	1.5		
80	146.0	145.3	143.4	--	--
	1.5	0.7	0.8		
320	147.1	145.6	144.1*	151.9**	145.4
	1.5	2.1	2.2	1.7	1.2

*$p<0.05$; **$p<0.01$ (Dunnett's t test)

58

007003

Creatinine(Mg/Dl) Mean/S.D.

Males	Treatment Period			Recovery Period	
Week--	4	8	13	14	18
Dose (ppm)					
0	0.872	0.700	0.795	0.732	0.846
	0.508	0.042	0.066	0.091	0.099
20	0.937†	1.016††	1.015††	--	--
	0.201	0.123	0.165		
80	0.741	0.718	0.835	--	--
	0.083	0.102	0.074		
320	0.854	0.904††	0.950†	0.847*	0.798
	0.080	0.133	0.104	0.105	0.078
Females					
0	0.785	0.711	0.809	1.131	1.212
	0.090	0.082	0.113	0.165	0.184
20	1.006**	0.935††	1.017**	--	--
	0.138	0.118	0.064		
80	0.842	0.761	0.842	--	--
	0.090	0.058	0.087		
320	1.035**	0.990††	1.063**	1.126	1.109
	0.056	0.150	0.142	0.089	0.149

Calcium(Mg/Dl)

Males					
0	9.46	9.33	10.71	9.07	8.39
	0.22	0.27	0.25	0.17	0.63
20	9.78	8.30**	10.12**	--	--
	0.24	0.52	0.40		
80	9.60	10.17**	10.87	--	--
	0.20	0.45	0.25		
320	9.41	9.13	10.33*	9.04	7.86*
	0.25	0.52	0.43	0.15	0.33
Females					
0	9.73	10.48	10.70	8.96	8.44
	0.19	0.17	0.31	0.29	0.68
20	9.25†	10.06*	10.44	--	--
	0.41	0.22	0.23		
80	9.44	10.47	10.30*	--	--
	0.36	0.33	0.27		
320	9.33†	10.48	10.23**	8.98	8.56
	0.38	0.43	0.36	0.25	0.94

*p<0.05; **p<0.01 (Dunnett's t test)

†p<0.05; ††p<0.01 (non-parametric--Kruskal-Wallis test)

59

66

007023

Urinalysis parameters

Volume
Specific gravity
pH
Protein
Sediment:
  RBC
  WBC
  Epithelial cells
  Calcium oxalate
  Triphosate
  Amorphous urate
  Uric acid
Ketones
Occult blood
Urobilinogen
Bilirubin
Glucose

Results: No treatment-related changes were evident.

POSTMORTEM EXAMINATIONS

Organ Weights

The following organs from every animal were weighed at the end of the treatment period:

Kidneys	Ovaries
Spleen	Testes
Liver	Brain
Heart	Adrenals

Results: Although not always statistically significant, the following measurements of liver weight were higher than respective control values in high-dose males and females:

a/ Percent body weight

p<0.01 for males and females

b/ Percent brain weight

p<0.01 for females; not statistically significant for males

c/ Absolute weight

p<0.05 for females; not statistically significant for males

Elevated liver weights were not observed in animals examined after the recovery period.

60

Liver Weights in Males and Females (grams)

Males	Mean/S.D.		
Dose (ppm)	Absolute Wt.	% Body Wt.	% Brain Wt.
0	11.6	2.86	570.1
	1.7	0.29	68.0
20	11.6	2.82	579.6
	1.4	0.20	71.9
80	12.2	2.94	591.0
	1.4	0.31	73.4
320	12.4	3.18††	612.6
	2.0	0.27	87.8
Females			
0	7.2	3.05	374.6
	0.5	0.21	32.7
20	7.3	3.14	392.1
	0.6	0.30	32.7
80	7.2	3.17	391.6
	1.1	0.32	45.1
320	8.0*	3.60††	432.3††
	0.7	0.29	30.3

* $p<0.05$: Dunnett's t test
†† $p<0.01$: non-parametric, Kruskal-Wallis test

Gross Necropsy

The gross examination performed at necropsy included external surfaces, all orifices, the cranial cavity, carcass; the brain, thoracic, abdominal and pelvic cavities, with associated organs and tissues, and the neck with associated tissues.

Results: No apparent treatment-related lesions were observed.

Histological Examination

The following tissues and organs from animals in every group were fixed in 4.0% formaldehyde for histopathologic processing:

All major lesions
Brain
Pituitary
Thyroids
Heart
Liver
Kidneys
Adrenals
Prostate
Seminal Vesicles
Testes with Epididymis
Spleen
Pancreas
Parathyroid
Urinary Bladder
Stomach (2 parts)
Small Intestine
Large Intestine
Lymph Nodes (cervical, mesenteric)
Uterus
Skeletal Muscle
Skin

61

Ovaries
Eyes (with optical nerve)
Tongue
Trachea
Esophagus
Salivary Glands
Thymus
Lungs
Sternum (with bone marrow)
Sciatic Nerve
Spinal Cord
Arteries
Aorta (thoracic)

Results: The only histologic changes that appeared to be associated with treatment were observed in the liver. Vacuolated hepatocytes that were predominantly centrilobular were observed in 1/15 mid-dose males and 6/15 high-dose males, but not in any other group. A distinct lobular pattern, associated with enlarged hepatocytes, was observed in 5/15 males and 4/15 females, all at the high-dosage level. This change was also observed in 1/15 control males and 2/15 mid-dose males. Other hepatic changes such as vacuolated hepatocytes, which were predominantly peripherolobular, and mononuclear focal mild hepatitis occurred at frequencies that were neither treatment- nor dosage related.

The only noteworthy change observed in kidneys was tubular calcification at the coritico-medullary junction which was observed in females with comparable frequency and degree in all groups.

The noted histologic changes in liver that were prevalent in high-dose rats were not observed in rats examined after the recovery period. However, the other noted changes in liver as well as kidney, which were observed with comparable frequency in all groups, were also seen in both control and dosed rats after the recovery period.

STATISTICAL ANALYSIS

The parameters evaluated by statistical analysis included body weights, food consumption, hematology, clinical chemistry, urinalysis and organ weights. The statistical methods used in this study are described on appended page 1.

CONCLUSIONS

The changes that occurred in rats receiving the 320 ppm dosage level of Cyproconazole, which appear to treatment-related, included inhibition of body weight gain, increased blood levels of sodium and creatinine with a concomitant decrease in calcium, increased liver weights and histological changes in liver. The noted changes in creatinine and calcium were also consistently observed in rats receiving the 20 ppm level but not in those at the mid-dose (80 ppm) level.

The inhibition of body weight gain was marginal at the high-dose level, however, it occurred in both sexes. The differences in mean terminal body weights between the control and high-dose groups were less than 10% for both sexes.

The observed changes in blood creatinine and calcium levels were detected in both sexes at the 8 and 13 week intervals, while increased sodium levels were seen at week 13 only. The investigator did not offer a possible explanation for the consistent changes seen in creatinine and calcium levels in the low- and high-dose groups, but not the mid-dose group. Since these changes were not observed during the recovery period, they should be considered treatment-related effects.

62

The histological changes observed in liver, which occurred predominantly in high-dose rats, are apparently related to treatment.

Since treatment-related changes were observed at the lowest dietary level of Cyproconazole administered (20 ppm), a NOEL was not attained in this study. Therefore, this study is not acceptable for regulatory purposes.

Core classification: minimum

TO 63

CYPROCONAZOLE Tox review 007003

Page 64 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007003

Reviewed by: K. Clark Swentzel K. Clark Swentzel 10/11/88
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. Marcia van Gemert 10/21/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 4-Week Feeding Study TOX. CHEM. NO.: 272E

MRID NO.: 406243-05

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): I.6158/84

SPONSOR: Sandoz Corp.

TESTING FACILITY: Sandoz Agrotoxicology

TITLE OF REPORT: SAN 619F: 4-Week Feeding Study in Rats (Supplemental report in support of 13-week feeding study in rats)

AUTHOR(S): C. Skinner

REPORT ISSUED: December 18, 1986

TEST DATES: May 2, 1984 - June 4-13, 1984

CONCLUSIONS: Male and female Han Wistar rats were administered Cyproconazole in the diet at levels of 10, 30, 100, 300 and 1000 ppm for 4 weeks. Evidently, this study was performed to determine the dietary levels that should be administered in a subsequent 13-week feeding study in rats. Changes associated with treatment, which were observed in males and females administered the highest dietary level, included decreased body weight and body weight gain, elevated ALT and LDH, increased absolute and relative (% body weight) liver weight and hepatocytomegaly. Elevated BUN and relative testes weights as well as liver vacuolation were observed in high-dose males. Increased cholesterol and relative adrenal weights were noted for high-dose females. The effects present in high mid-dose (300 ppm) males and females were: elevated LDH, increased absolute and relative liver weight and liver vacuolation. Increased relative testes and adrenal weights were seen in high mid-dose males and females, respectively. The LEL and NOEL were 300 and 100 ppm, respectively.

Core classification: supplementary data (not a Guideline Study)

Quality assurance statement: Signed and dated

65

007003

TEST MATERIAL

Concentration of technical material = 95.7%; Lot no.: 8404; Code name: SAN 619 F; Isomer composition: 619 A = 54.3%, 619 B = 41.4%; Description: white powder.

TEST ANIMAL DATA

Rats--Han Wistar; Age at initiation of study = 11 weeks; Body weights at study iniation: males = 265 ± 5.7g; females = 193 ± 1.4g; Identification: individual numbers.

Group assignment: animals were randomly selected and 16 rats/sex were assigned to 1 control and 5 dosage groups for the 4-week dosage period.

Acclimation: 5 days

Housing: Individually in plastic cages

Food: Kliba, powdered diet no. 21-343-4: ad libitum

Water: municipal tap water: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 23 ± 2°C; Relative humidity - 50 ± 20%.

METHODS

Administration of test material

The test material was mixed with the diet from a 1% premix at levels of 10, 30, 100, 300 or 1000 ppm which was fed to the test animals for 4 weeks. The frequency of test diet preparation was not indicated.

Test Diet Analysis

Cyproconazole levels in the premix and final diets were analysed prior to study initiation and at 2 and 4 weeks by the registrant.

Results: The mean concentrations of Cyproconazole (3 analyses) were:

Dose(ppm)	Mean concentration(ppm)	Percent of nominal concentration
10	10.1	101.0
30	26.7	89.0
100	81.0	81.0
300	265.0	88.3
1000	933.3	93.3

Symptoms and Mortality

The investigator indicated that examinations included daily neurological, oral

66

behavioral inspections. 007003

Results: No mortalities or drug-related symptoms were observed. Food wastage was observed among high-dose animals.

Body Weight

Measured on day 1 and weekly thereafter.

Results: Body weights were decreased in high-dose males and females throughout the study. Body weights were also decreased in high-mid-dose males and females during weeks 1 and 2, however, they were comparable to respective control values at study termination. Body weight gain was also decreased in high-dose males and females.

Mean body weights(a) 0-4 weeks

Dose(ppm)	0	10	30	100	300	1000
Males	321	316	309	316	308	279††
Rel.to Cont.	---	-1.6%	-3.7%	-1.6%	-4.0%	-13.1%
Females	209	212	210	211	203	195††
Rel.to Cont.	---	+1.4%	+0.5%	+0.9%	-2.9%	-6.7%

Mean body weight gain(a) 0-4 weeks

Males	21.5	23.0	20.2	22.6	20.7	13.3††
Rel.to Cont.	---	+7.0%	-6.0%	+5.1%	-3.7%	-38.1%
Females	7.2	7.8	7.7	7.3	5.8	3.2††
Rel.to Cont.	---	+8.3%	+6.9%	+1.4%	-19.4%	-55.6%

†† $p<0.01$ (non-parametric: Kruskal-Wallis test)

Food Consumption

Determined weekly.

Results: The only differences from controls were noted in high-dose females, among which food consumption increased during weeks 2(16.7%), 3(28.7%) and 4(47.8%). All of these increases were statistically significant ($p<0.01$, Kruskal-Wallis test). These data are not consistent with the body weight above; the investigator did not indicate if wastage was accounted for in these calculations.

Compound Consumption

The investigator computed 4-week compound consumption values based on food consumption data and measured dietary levels of compound.

67

Accumulative (four-week) Phorate consumption (mc/kg/day)

Dose (ppm)	Males	Females
10	0.80	0.93
30	2.29	2.94
100	8.14	9.81
300	25.32	31.54
1000	96.18	127.59

Laboratory Investications

Hematology and clinical chemistry were determined on 8 rats/sex/group. Rats used at 2 weeks were not used again at 4 weeks. The animals were fasted for 24 hours prior to collection of blood, which was drawn from the sublingual vein.

Hematology parameters

Hematology parameters

Hematocrit-Hct
Hemoglobin-Hgb
Erythrocytes-RBC
Mean cell volume-MCV
Mean corpuscular hemoglobin-MCH
Mean corpuscular hemoglobin concentration-MCHC
Leukocytes (total and differential)-WBC
Platelets
Reticulocytes

Results: A statistically significant decrease in the hematocrit was observed in high-dose males at 2 weeks and in low medium-dose, high medium-dose and high-dose males at 4 weeks. The biological significance of this change is doubtful since the values were not dose-related and it occurred in only one sex.

Hematocrits (%)in Male Rats

Weeks / Dose(ppm)--	0	10	30	100	300	1000
			Mean/S.D.			
2	48.25 1.00	47.01 1.33	46.99 1.06	48.54 1.57	47.83 1.41	46.36* 1.34
4	49.79 2.02	49.48 1.52	47.43* 1.51	48.86 1.45	46.85** 1.38	47.25* 2.04

* $p<0.05$, ** $p<0.01$ (Dunnett's t test)

Clinical chemistry parameters

Hemolytic score	Calcium
Glucose	Chloride
Blood urea nitrogen	Total cholesterol
Bilirubin	Glutamic pyruvic transaminase(SGPT)-ALT
Albumin	Glutamic oxaloacetic transaminase(SGOT)-AST
Total protein	Alkaline phosphatase
Sodium	Creatinine
Potassium	Lactate dehydrogenase(LDH)

Results: Elevated levels of blood urea nitrogen were statistically significant in high-dose males at both intervals and in high-dose females at 2 weeks. Cholesterol was increased in high-dose females and an increased trend was evident in males. Increased ALT was observed in high-dose males and females, however, the increase in females at week 2 was not statistically significant. LDH was increased in high mid- and high-dose males and females; the increase in high-dose females at week 4 was not statistically significant, however, there was 1 low value in this group. Bilirubin was decreased in high mid-dose and high-dose males at week 4 and in corresponding females at both intervals but only the the change in high-dose females at week 4 was statistically significant. It is evident from these data that compound-induced clinical chemistry changes occurred in the high mid- and high-dose groups.

Clinical Chemistry Changes

Week Dose(ppm)--	0	10	30	100	300	1000
			Mean/S.D.			
Blood urea nitrogen(Mg/Dl)						
Males						
2	14.59	14.68	17.23	15.89	16.93	17.81†
	1.76	2.04	4.30	1.94	1.70	1.58
4	14.73	14.40	15.10	14.49	16.56	16.80†
	1.72	0.97	1.56	2.71	2.12	3.06
Females						
2	16.05	15.65	17.05	17.19	17.36	20.13†
	1.57	1.66	1.99	1.62	2.27	2.87
4	18.44	15.53	18.56	19.55	16.34	18.83
	4.55	3.34	2.58	3.87	2.50	4.05
Cholesterol(Mg/Dl)						
Males						
2	71.0	69.3	63.0	74.5	82.8	89.4
	9.5	15.8	10.0	12.2	14.6	9.3
4	64.3	66.3	69.9	73.9	72.4	82.6
	11.6	9.9	7.5	18.5	12.0	15.3
Females						
2	55.4	66.6	58.5	70.5	71.6	97.8††
	9.7	6.6	12.3	14.2	16.6	23.8
4	61.1	69.0	63.1	66.1	77.8	104.0††
	9.1	10.1	13.9	16.2	9.9	20.9

† $p<0.05$, †† $p<0.01$ (non-parametric--Kruskal-Wallis test)

69

Clinical Chemistry Changes(cont.)

Week Dose(ppm)--	0	10	30	100	300	1000
			Mean/S.D.			
ALT(IU)						
Males						
2	24.5	28.6	25.5	26.5	25.6	42.6**
	2.0	3.3	1.6	2.7	2.9	6.7
4	28.0	24.1	22.9*	24.0	24.3	41.6**
	3.8	3.0	3.4	3.3	3.5	4.8
Females						
2	18.6	21.1	19.8	19.0	20.8	23.1
	3.2	5.9	3.4	3.4	2.2	2.4
4	19.4	18.3	19.3	19.3	18.5	24.5*
	2.7	3.0	2.8	2.4	4.2	3.9
LDH(IU)						
Males						
2	108.4	121.8	189.0	271.8††	158.6	203.0†
	14.5	51.2	89.8	149.3	63.9	39.9
4	182.3	206.8	174.1	182.1	323.0*	400.5**
	124.9	106.0	44.7	55.8	152.5	97.3
Females						
2	118.3	118.8	135.0	127.5	182.8**	266.9**
	29.7	37.3	28.6	39.8	43.4	43.0
4	173.6	144.1	198.9	157.1	222.5	304.0
	58.0	82.1	109.1	25.7	25.0	89.5
Bilirubin(Mg/Dl)						
Males						
2	0.114	0.111	0.099	0.108	0.120	0.125
	0.043	0.023	0.064	0.050	0.031	0.092
4	0.511	0.478	0.490	0.450	0.456	0.435
	0.299	0.320	0.322	0.321	0.277	0.285
Females						
2	0.160	0.091	0.105	0.109	0.124	0.148
	0.085	0.053	0.023	0.042	0.037	0.085
4	0.243	0.245	0.229	0.231	0.180	0.136**
	0.060	0.068	0.057	0.046	0.040	0.051

* $p<0.05$, ** $p<0.01$ (Dunnett's t test); † $p<0.05$, †† $p<0.01$ (Kruskal-Wallis test)

007003

Urinalysis parameters

Volume		Ketones
Specific gravity		Occult blood
pH		Urobilinogen
Protein		Bilirubin
Sediment:		Glucose
RBC	Triphosate	
WBC	Amorophous urates	
Epithelial cells	Uric acid	
Calcium oxalate		

Results: The mean values indicate that there was a trend towards increased calcium oxalate in dosed males and increased amorphous urate in high-dose males and females, however, an examination of the individual data showed these increases were influenced by sporadic high values and/or abnormally low control values. Therefore, these changes do not appear to be compound-related.

POSTMORTEM EXAMINATIONS

Organ Weights

The following organs from every animal were weighed at the end of the treatment period:

Kidneys	Ovaries
Spleen	Testes
Liver	Brain
Heart	Adrenals

Results: Absolute and relative (% body wt.) liver weights were increased in high mid- and high-dose males and females. Relative testes and adrenal weights were also increased in high mid- and high- dose males and females, respectively.

Absolute and Relative Liver Weights

Dose(ppm)	0	10	30	100	300	1000
			g(Mean/S.D.)			
Males						
Absolute	11.0	11.4	11.3	11.7	12.3	15.6^{TT}
	0.9	1.1	1.4	1.2	1.6	1.9
Relative	3.14	3.27	3.33	3.36	3.64^{T}	5.09^{T}
(% BW)	0.22	0.32	0.34	0.25	0.33	0.57
Females						
Absolute	7.08	7.04	7.25	7.73	7.97**	9.56**
	0.81	0.90	0.73	0.76	0.63	0.87
Relative	3.30	3.23	3.32	3.58	3.79^{TT}	4.78^{TT}
(% BW)	0.38	0.36	0.27	0.37	0.28	0.42

* $p<0.05$, ** $p<0.01$(Dunnett's t test); ^{T} $p<0.05$, ^{TT} $p<0.01$(Kruskal-Wallis test)

78

11

Organ Weights (cont.)

Dose(ppm)	0	10	30	100	300	1000
			g(Mean/S.D.)			
Relative weights (% BW)						
Testes	0.93 0.05	0.94 0.13	0.97 0.08	1.01 0.11	1.02† 0.09	1.10† 0.09
Adrenals (females)	0.041 0.007	0.040 0.007	0.042 0.004	0.042 0.006	0.047†† 0.006	0.045† 0.005

† $p<0.05$, †† $p<0.01$ (Kruskall-Wallis test)

Gross Necropsy

The investigator indicated that a complete gross examination was performed on all animals that died spontaneously or were killed in a moribund condition. The examination included external surfaces; all orifices; the cranial cavity; carcass; brain; the thoracic, abdominal and pelvic cavities with associated organs and tissues; and the neck with associated tissues.

Results: Changes in liver described as visible architecture with white nodules and hyperemia was observed in high-dose males (4/16) only.

Histological Examination

The following tissues and organs from animals in every group were fixed in 4.0% formaldehyde for histopathologic processing:

All major lesions
Brain
Pituitary
Thyroids
Heart
Liver
Kidneys
Mammary Gland
Adrenals
Prostate
Seminal Vesicles
Testes with Epididymis
Ovaries
Eyes (with optical nerve)
Tongue
Trachea
Esophagus
Thymus
Lungs
Spleen
Pancreas
Parathyroid
Urinary Bladder
Stomach (2 parts)
Duodenum
Jejunum
Ileum
Colon
Rectum
Lymph Nodes (cervical, mesenteric)
Uterus
Skeletal Muscle
Skin
Sternum (with bone marrow)
Sciatic Nerve
Spinal Cord (cervical/thoracic)
Aorta (thoracic)
Salivary Glands

12

Results: Tubular calcification was observed in the kidneys of females at comparable rates in all groups; no relationship to treatment was apparent. The only obvious treatment-related effects were observed in liver; increased incidences of vacuoles, attributed to reversible storage, were present in high mid-dose males and females and high-dose males. Hepatocytomegaly, predominantly centrilobular, was noted in high-dose males and females. Focal mononuclear hepatitis was present in some treatment groups but the incidence was not dosage-related.

Histopathologic Changes in Liver(16/sex/group examined)

Dose(ppm)	0	10	30	100	300	1000
Vacuoles						
Males	3	0	0	0	13	15
Females	0	1		3	7	1
Hepatocytomegaly						
Males	0	0	0	0	0	10
Females	0	0	0	0	0	2
Hepatitis (Mononuclear)						
Males	0	4	2	4	2	0
Females	0	4	5	3	3	0

Statistical Analysis

Body weight, body weight change, food consumption, substance intake, hematology, clinical chemistry, urinalysis, organ weights and organ weight/body weight ratios were analysed statistically for differences between treated and control group mean values. The statistical methods used in this study are described on appended page 1.

Discussion and Conclusions

Evidently this study was performed to determine the appropriate dosage levels to administer in a 13-week feeding study in rats (MRID 406243-04). Although the investigator indicated that this is a supplemental report in support of the 13-week study, the data in this report do not change the conclusion that a NOEL was not attained in that study.

Dosage-related changes were observed in high-(1000ppm) and high mid-(300ppm) dose rats. Changes present in both high-dose males and females were: decreased body weight and body weight gain, elevated ALT, LDH and absolute/relative liver weights, decreased bilirubin and hepatocytomegaly. Changes in high-dose males only were: increased BUN, increased relative testes weight and liver vacuolation and in high-dose females: decreased food consumption, elevated cholesterol and increased

13

relative adrenal weight. The effects present in high mid-dose males and females were: elevated LDH, increased absolute and relative liver weight and liver vacuolation. Increased relative testes and adrenal weights were seen in high mid-dose males and females, respectively.

The LEL and NOEL in this study were 300 and 100 ppm, respectively.

Core classification: supplementary data (not a guideline study)

74

CYPROCONAZOLE Tox review 007003

Page 75 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel K. Clark Swentzel 10/20/88 007003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. James W. Rowe 11/16/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 13-Week Feeding Study TOX. CHEM. NO.: 272E

MRID NO.: 406077-19

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 6521/86

SPONSOR: Sandoz Corp.

TESTING FACILITY: Research and Consulting Company, A.G., Itingen, Switzerland

TITLE OF REPORT: 13-Week Feeding Study in Beagle Dogs

AUTHOR(S): S. Warren, S. Carpy, C. Skinner & J. Karapally

REPORT ISSUED: December 12, 1986

TEST DATES: April 17, 1985 - July 23 & 24, 1985

CONCLUSIONS

Male and female beagle dogs were administered Cyproconazole in the diet at levels of 20, 100 and 500 ppm for 13 weeks. Changes associated with treatment, observed in both sexes administered the highest dietary level, included "slack muscle tone", inhibited body weight gain, increased platelet counts, decreased: bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein and albumin and increased alkaline phosphatase and gamma glutamyl transferase; decreased food consumption was seen in high-dose males. Increased absolute and relative liver weights and increased relative kidney weights were noted for high-dose males and females; relative brain weights were increased in high-dose females. Histopathologic evidence of liver toxicity in high-dose males and females included hepatocytomegaly, degeneration of single hepatocytes and cytoplasmic inclusions. Evidence of liver toxicity in mid-dose dogs was increased absolute liver weights in males and hepatocytomegaly in males and females.

The LEL in this study, based on adverse effects in liver, was 100 ppm (approximately 4 mg/kg/day and the NOEL was 20 ppm (approximately 0.8 mg/kg/day).

Since this study was not inspected by a QAU during the in-life phase, a data audit, signed and dated by a QA Officer, must be submitted to the Agency before this study can be accepted for regulatory purposes.

Core classification: supplementary (can be upgraded to minimum provided an acceptable QAU audit is provided as noted above)

Quality assurance statement: Signed and dated by the Study Director (NOT ACCEPTABLE)

83
76

TEST MATERIAL

Concentration of technical material = 95.6%; Lot no.: 8507; Code name: SAN 619 F; Isomer composition: not reported; Description: white powder.

TEST ANIMAL DATA

Dogs--Beagle; Age at initiation of study = 7 months; Body weights at study initiation: males = 9.8 ± 0.8 kg; females = 8.5 ± 0.9 kg; Identification: individual numbers.

Group assignment: animals were randomly selected and 4 dogs/sex were assigned to 1 control and 3 dosage groups for the 13-week dosage period.

Acclimation: 8 weeks

Housing: Individually in hanging steel mesh self-cleaning cages

Food: Kliba, powdered diet no. 24-335-1: 500g provided per day

Water: municipal tap water: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 22 ± 2°C; Relative humidity - 55 ± 10%.

METHODS

Administration of test material

The test material was mixed with the diet from a 1% premix at levels of 20, 100 or 500 ppm which was fed to the test animals for 13 weeks. The premix was prepared monthly and stored at 3-5°C. The frequency of diet preparation was not indicated.

Test Diet Analysis

Cyproconazole levels in the premix and final diets were analysed prior to study initiation and subsequently at monthly intervals by the registrant.

Results: The mean concentrations of Cyproconazole (Weeks: 1,4,8 & 13) were within acceptable limits (± 15% of nominal).

Dose(ppm)	Mean concentration(ppm)	Percent of nominal concentration
20	20.9	104.5
100	92.8	92.8
500	457.5	91.5

77

Symptoms and Mortality

The dogs were examined twice daily for treatment-induced symptoms. The examinations included oral, behavioral, color, feces and urine inspections.

Results: All dogs survived until the scheduled terminal sacrifice. The investigator indicated that "slack muscle tone" was observed in all high-dose animals. This change was first observed in week 1 and all dogs were affected after 2 weeks of treatment. This effect persisted until the end of the study in 3/4 males and 2/4 females. No other relevant clinical changes were noted.

Ophthalmoscopy

Control and high-dose animals were examined prior to the commencement of treatment and prior to termination using a fundus camera following administration of a mydriatic.

Results: No lesions were detected.

Body Weights

Individual body weights were recorded weekly.

Results: Body weight gain was inhibited in high-dose males and females. Mean terminal body weights of dogs in this group were -12.8 and -10.5% for males and females, respectively, relative to corresponding control weights.

Body weight gain

Interval(weeks)

Dose(ppm)	0	20	100	500
	Kg/% difference(rel. to control)			
Males				
0 - 6	1.4	1.4	1.6	0.5
	---	0.0	+14.3	-64.3
6 - 13	0.7	1.0	0.8	0.9
	---	+42.9	+14.3	+28.6
0 - 13	2.1	2.4	2.4	1.4
	---	+14.3	+14.3	-33.3
Females				
0 - 6	0.7	1.0	0.7	0.3*
	---	+42.9	0.0	-57.1
6 - 13	0.5	0.9	0.6	0.2
	---	+80.0	+20.0	-60.0
0 - 13	1.2	1.9*	1.3	0.5*
	---	+58.3	+8.3	-58.3

* $p<0.05$ (no statistical analysis performed on data tabulated for weeks 6 - 13)

Food Consumption

Determined twice weekly and expressed as grams of food consumed per dog per day.

Results: Food consumption was slightly lower in high-dose males throughout the study. During the first 6 weeks, consumption was 19% lower and during the last 7 weeks, 11% lower, in comparison to controls. Food consumption values in the other treatment groups were comparable to respective control values for both males and females.

Calculated Intake of SAN 619 F

Individual compound consumption values were calculated at weekly intervals from individual bodyweight and food consumption data and nominal dietary concentrations of SAN 619 F.

Results: The investigator calculated the following mean compound consumption values for each group:

SAN 619 F intake values

Dose (ppm)	Males		Females	
	mean	max.	mean	max.
	(mg/kg/day)			
20	0.77	0.95	0.70	0.83
100	4.00	4.59	3.25	3.96
500	18.18	19.76	19.17	21.95

Laboratory Investigations

Blood profiles and clinical chemistry values were determined for all dogs. The animals were fasted for approximately 16 hours prior to collection of venous blood samples. Hematology parameters were measured at weeks 0, 4, 8 and 13. Clinical chemistry determinations were made at weeks -1, 0, 4, 8, 12 and 13, unless indicated otherwise. These determinations included all of the parameters stipulated in the Subdivision F Guidelines.

Hematology parameters

Hematocrit-Hct
Hemoglobin-Hgb
Erythrocytes-RBC
Mean cell volume-MCV
Mean corpuscular hemoglobin-MCH
Mean corpuscular hemoglobin concentration-MCHC
Leukocytes (total and differential)-WBC
Platelets
Reticulocytes

Results: Increased platelet counts were observed in high-dose males and females only. These counts exceeded control as well as baseline values at all post-treatment intervals.

Platelet counts (high-dose vs. control)

Dose (ppm) / Week--	0	4	8	13
		Mean/S.D. ($10^3/mm^3$)		
Males				
0	299.5 56.0	292.5 78.6	292.5 85.0	253.5 57.0
20	282.3 95.6	306.8 43.3	279.3 14.4	267.3 22.1
100	351.8 36.7	358.0 43.1	329.3 50.2	296.0 42.2
500	333.5 37.0	410.8 72.7	429.0 110.2	442.3** 116.5
Females				
0	348.8 111.7	324.3 94.3	316.8 86.9	310.0 83.5
20	284.3 93.3	276.8 71.9	276.3 82.0	284.3 87.3
100	279.5 53.6	287.5 32.3	279.5 54.8	275.0 47.2
500	334.3 78.0	437.5 61.9	456.5* 58.3	427.5 66.6

* $p<0.05$; ** $p<0.01$ (relative to controls)

Clinical chemistry parameters

	Test Interval (weeks)				
	-1	0	4	8	13
Hemolytic score	x	x	x	x	x
Glucose	x	x	x	x	x
Urea	x	x	x	x	x
Total Bilirubin	x	x	x	x	x
Albumin	x	x	x	x	x
Total protein	x	x	x	x	x
Sodium	x	x	x	x	x
Potassium	x	x	x	x	x
Calcium	x	x	x	x	x
Chloride	x	x	x	x	x
Total Cholesterol				x	x
High Density Lipoprotein-Cholesterol(HDL-Chol)				x	x
Triglycerides		x	x	x	x
Glutamic pyruvic transaminase(SGPT)-ALT	x	x	x	x	x
Glutamic oxalpacetic transaminase(SGOT)-AST	x	x	x	x	x
Gamma glutamyl transferase(GGT)	x	x			x
Glutamate dehydrogenase(GLDH)	x	x			x
Leucine aryl amidase(LAP)	x	x			x
Alkaline phosphatase	x	x	x	x	x
Creatinine	x	x	x	x	x
Lactate dehydrogenase(LDH)	x	x	x	x	x
Inorganic Phosphorous	x	x	x	x	x
Creatinine phosphokinase(CPK)				x	x
Cortisol					x
Testosterone					x

Results:

Statistically significant differences in treatment-related changes, in comparison to controls and/or baseline data, are indicated in the following tabulated data as follows: 1) comparison to controls (* $p<0.05$, ** $p<0.01$ - parametric test; † $p<0.05$, †† $p<0.01$ - non-parametric test); 2) comparison to baseline (§ $p<0.05$, §§ $p<0.01$ - parametric test).

Changes in clinical chemistry parameters observed in high-dose dogs, which appeared to be treatment-related, included decreases in bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein and albumin and increases in alkaline phosphatase and GGT. Other fluctuations noted in high-dose animals, which did not appear to be related to treatment because they were not dose-related and/or were comparable to baseline values, included increases in creatinine, ALT, CPK, GLDH, cortisol and testosterone as well as decreases in calcium and inorganic phosphorous.

Although not always statistically significant, the noted treatment-related changes were observed in both sexes at the 4, 8 and 13 week intervals with the exception of decreased cholesterol (total and HDL), observed at weeks 8 and 13 (only intervals for measurement) and increased GGT at week 13 (measured at weeks -1, 0 and 13).

Treatment-related changes in clinical chemistry parameters

Dose(ppm) Weeks--	-1	0	4	8	13
			Mean/S.D.		
Bilirubin(uMol/L)					
Males					
0	0.428	1.323	0.818	1.145	1.70[a]
	0.375	0.106	0.401	0.232	0.30[illegible]
20	1.207	1.340	1.018	1.233	1.640
	0.339	0.155	0.362	0.372	0.252
100	0.828	1.048	0.825	1.045	1.635
	0.577	0.349	0.567	0.235	0.267
500	1.205	1.250	0.575	0.785[§]	1.248
	0.512	0.225	0.443	0.395	0.415
Females					
0	0.548	1.255	1.035	1.155	1.625
	0.409	0.385	0.826	0.394	0.249
20	0.993	1.733	1.250	1.453	2.073
	0.574	0.655	0.494	0.582	0.470
100	0.858	1.700	1.130	1.440	1.975
	0.573	0.254	0.733	0.385	0.168
500	0.950	1.570	0.518[§]	0.673[§]	1.303
	0.478	0.135	0.602	0.360	0.262
Total cholesterol(MMol/L)					
Males					
0	--	--	--	4.195	3.968
				0.599	0.802
20	--	--	--	4.310	4.360
				0.221	0.311
100	--	--	--	4.510	4.468
				0.700	0.932
500	--	--	--	2.043[**]	2.078[**]
				0.439	0.479
Females					
0	--	--	--	3.443	3.828
				0.327	0.676
20	--	--	--	5.713[*]	6.153[**]
				1.604	0.974
100	--	--	--	4.185	4.348
				0.408	0.300
500	--	--	--	2.563	2.793
				0.728	0.607

007003

Treatment-related changes in clinical chemistry parameters

Dose(ppm) Weeks--	-1	0	4	8	13
			Mean/S.D.		
HDL-Cholesterol(MMol/L)					
Males					
0	--	--	--	4.565 0.872	4.288 0.747
20	--	--	--	4.605 0.536	5.429 1.002
100	--	--	--	4.813 0.895	4.560 0.660
500	--	--	--	2.280** 0.544	2.908 0.389
Females					
0	--	--	--	3.648 0.334	4.638 0.591
20	--	--	--	6.343** 1.456	5.223 1.639
100	--	--	--	4.468 0.640	3.245 1.591
500	--	--	--	2.820 0.722	2.568 0.527
Triglycerides(MMol/L)					
Males					
0	--	0.453 0.099	0.500 0.073	0.443 0.132	0.373 0.075
20	--	0.583 0.099	0.565 0.114	0.495 0.116	0.443 0.053
100	--	0.413 0.032	0.418 0.021	0.420 0.068	0.383 0.039
500	--	0.465 0.060	0.258† 0.022	0.313 0.040	0.300 0.048
Females					
0	--	0.378 0.019	0.485 0.095	0.478 0.085	0.400 0.048
20	--	0.568* 0.053	0.620 0.076	0.563 0.159	0.565 0.118
100	--	0.475 0.051	0.468 0.061	0.440 0.081	0.505 0.102
500	--	0.513* 0.120	0.243** 0.072	0.335 0.146	0.365 0.123

83

Treatment-related changes in clinical chemistry parameters

Dose(ppm) Weeks--	-1	0	4	8	13
			Mean/S.D.		
Total Protein(G/L)					
Males					
0	49.92 4.82	59.22 2.61	58.72 3.15	53.05 2.83	60.93$^{\$}$ 2.44
20	44.03 10.61	58.18 3.24	61.63 2.41	55.40 2.15	62.45$^{\$}$ 2.85
100	52.45 1.58	57.85 2.06	59.93 2.80	54.13 2.51	63.85$^{\$\$}$ 1.42
500	51.23 4.18	58.35 2.34	55.25 1.41	49.90 1.28	57.42$^{\$}$ 3.48
Females					
0	54.00 4.30	59.63 3.32	61.07 3.53	55.15 3.16	62.20$^{\$}$ 2.15
20	53.30 3.11	57.92 2.40	62.38 1.76	56.05 1.60	63.80$^{\$}$ 2.04
100	52.75 1.92	57.57 0.69	60.38 2.82	54.55 2.65	64.22$^{\$}$ 3.10
500	54.60 1.25	57.78 2.98	54.85* 4.04	49.52$^{*\$}$ 3.67	59.27 6.90
Albumin(G/L)					
Males					
0	32.40 1.69	29.68 0.83	30.10 1.24	30.48 0.85	30.78 0.90
20	30.07 1.17	28.43 1.51	29.83 1.53	30.35 1.50	29.75 1.67
100	31.78 0.94	28.35 1.76	28.10 1.32	28.58$^{\dagger\$}$ 1.33	28.78$^{\$}$ 0.68
500	32.10 2.07	29.38 1.18	24.58** 1.24	24.10$^{\dagger\$\$}$ 2.60	23.45$^{**\$\$}$ 2.79
Females					
0	33.38 0.96	29.53 1.14	30.60 0.53	31.93$^{\$}$ 0.67	30.70$^{\$\$}$ 0.24
20	33.78 2.48	29.33 1.50	31.68 1.58	33.13 1.64	31.45 2.33
100	33.30 2.50	28.58 2.41	30.58 1.85	31.10$^{\$}$ 1.93	29.95$^{\$}$ 2.12
500	32.38 1.82	28.30 1.31	24.25** 1.55	24.43$^{**\$\$}$ 1.70	23.95$^{\dagger\$\$}$ 2.30

84

~~91~~

Treatment-related changes in clinical chemistry parameters

Dose(ppm) Weeks--	-1	0	4	8	13
			Mean/S.D.		
Alkaline Phosphatase(U/L)					
Males					
0	200.00	180.50	142.25	121.50$	38.62$$
	23.51	21.08	10.05	21.02	7.51
20	216.33	206.25	158.25†	167.00	137.67
	34.70	23.89	34.65	46.31	42.00
100	191.50	188.75	151.25	132.75$	119.25$
	12.50	34.40	26.74	34.07	33.59
500	213.25	201.25	394.75†	447.25†	459.25**
	28.43	35.08	125.99	175.05	161.50
Females					
0	219.00	210.00	181.25	149.75$	118.85$$
	43.89	39.10	49.34	29.03	25.69
20	181.75	166.75	138.50	132.05$	99.15$$
	35.80	31.14	25.65	42.26	22.99
100	260.00	347.00	230.00	188.80$	156.05$
	101.72	93.83	62.69	6361	54.80
500	231.50	209.75	349.00**	466.50†$	456.00**$$
	62.94	58.70	63.68	139.85	97.83
Gamma glutamyl transaminase(U/L)					
Males					
0	2.180	0.983	--	--	2.755
	0.176	0.322			0.357
20	1.557	1.323	--	--	2.238
	0.630	0.202			0.384
100	1.405	1.218	--	--	2.785$$
	0.407	0.463			0.425
500	1.905	1.763	--	--	4.330†$$
	0.523	0.740			0.989
Females					
0	2.110	1.148	--	--	2.413
	0.600	0.478			0.377
20	1.123	0.953	--	--	2.433$$
	0.266	0.573			0.149
100	1.793	1.273	--	--	4.085†$$
	0.207	0.633			0.816
500	1.375	1.718	--	--	4.133†$$
	0.956	0.730			0.374

Urinalysis

Urine samples were collected over a 4 hour period at each interval (-2, 4, 8 and 13 weeks), during which time food and water were withheld. The following parameters were investigated immediately after each collection period:

Urinalysis parameters

Volume
Specific gravity
pH
Protein
Sediment:
- RBC
- WBC
- Epithelial cells
- Calcium oxalate
- Triphosate
- Amorphous urate
- Uric acid

Ketones
Occult blood
Urobilinogen
Bilirubin
Glucose

Results: There were no noted changes in the investigated urinary parameters indicative of a compound-related effect.

Organ Weights

The following organs were excised, trimmed and weighed:

Kidneys
Liver
Testes/Ovaries
Adrenals
Brain
Thyroids

The investigator indicated that organ weight to bodyweight and organ weight to brain weight ratios were calculated.

Results: It is apparent from the individual data that organ to body weight ratios were calculated, however, only summarized data for absolute organ weights in males and females and organ to body weight ratios for females were submitted. Also, calculated organ to brain weight ratios were not submitted.

Absolute liver weights were increased in mid- and high-dose males and high-dose females. Relative weights (% body weight) for brain, liver and kidneys were increased in high-dose females. Relative liver and kidney weights were also increased in males.

Liver weights — Mean/S.D.

Dose (ppm)	Males		Females	
	Absolute wt. (g)	Relative wt.† (% B.W.)	Absolute wt. (g)	Relative wt. (% B.W.)
0	307.7	2.6	261.1	2.9
	46.4	0.3	20.5	0.1
20	315.7	2.5	293.3	2.8
	38.6	0.4	16.2	0.4
100	386.6	3.3	265.2	2.8
	34.7	0.4	25.1	0.2
500	440.9**	4.3	348.4**	4.4**
	55.9	0.5	48.0	0.3

* $p<0.05$, ** $p<0.01$; † Statistical analysis not performed

Kidney weights — Mean/S.D.

Dose (ppm)	Males		Females	
	Absolute wt. (g)	Relative wt.† (% B.W.)	Absolute wt. (g)	Relative wt. (% B.W.)
0	54.27	0.46	37.89	0.43
	14.75	0.11	1.77	0.05
20	56.94	0.44	42.41	0.40
	6.84	0.05	3.43	0.01
100	56.88	0.48	39.12	0.41
	10.70	0.06	2.73	0.03
500	51.55	0.51	38.95	0.49*
	7.94	0.08	2.36	0.02

Relative brain weights (% B.W.)

	Males†	Females
0	0.70	0.90
	0.00	0.10
20	0.65	0.80
	0.06	0.10
100	0.70	0.80
	0.08	0.10
500	0.73	1.00**
	0.05	0.10

* $p<0.05$, ** $p<0.01$, † Statistical analysis not performed

Macroscopic Examination

This procedure included examination of external surfaces, all orifices, the cranial cavity, the carcass; the brain, thoracic, abdominal and pelvic cavities with associated organs and tissues as well as the neck with its tissues.

Results: No treatment-related changes were noted.

Histopathologic Examination

Representative samples of all of the following organs, tissues and anomalies from all animals were fixed in 4% formaldehyde for histopathological processing.

All major lesions
Brain
Pituitary
Thyroid
Heart
Liver
Kidneys
Adrenals
Prostate
Thymus
Testes with Epididymis
Gall Bladder
Spleen
Pancreas
Lungs
Urinary Bladder
Lymph Nodes (cervical, mesenteric)
Skin
Uterus
Skeletal Muscle
Stomach (2 parts)
Duodenum
Jejunum
Ileum

Mammary Gland	Cecum
Sternum (w.bone marrow)	Colon
Cervix	Rectum
Ovaries	Bone Marrow (femoral)
Eyes (with optical nerve)	Sciatic Nerve
Tongue	Spinal Cord(cervical/thoracic)
Trachea	Arteries
Esophagus	Aorta (thoracic)
Salivary Glands	Parathyroid

Results: The only microscopic changes that appear to be related to treatment were observed in the liver of males and females of the mid- and high-dose groups. Hepatocytomegaly occurred at a frequency of 2/4 for both mid-dose males and females and 4/4 for both high-dose males and females. Also, degeneration of single hepatocytes and cytoplasmic inclusions were each observed at a rate of 1/4 at the high-dose in both males and females. Other noted changes occurred sporadically or were observed at comparable frequencies between groups and could not conclusively be associated with treatment.

Statistical Analysis

Body weight, food consumption hematology, clinical chemistry, urinalysis, organ weight and organ weight/body weight ratios were analysed for differences between treated and control group mean values. Intra-group differences between pre- and post treatment mean values were also analysed, where applicable. A description of the methodology for the statistical analyses used in this study is shown on appended page 1.

Conclusions

Compound-related effects, which were evident among males and females in the high-dose group, were reflected by changes in several of the investigated parameters. During the clinical observations, the investigator indicated that these animals had "slack muscle tone" throughout the study. Although body weight gain was inhibited in both sexes, food consumption was decreased among males only. The clinical laboratory investigations revealed the following changes among high-dose males and females: increased platelet counts; decreased bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein and albumin (primary cause of decreased total protein) and increased alkaline phosphatase and gamma glutamyl transferase(GGT). Group organ weight data showed increased absolute and relative (% body weight) liver weight and increased relative kidney weight in high-dose males and females and increased relative brain weight in high-dose females. Histopathologic data revealed liver changes which included hepatocytomegaly, degeneration of single hepatocytes and cytoplasmic inclusions.

The only apparent compound-related effects observed in mid-dose animals were changes in liver which included increased absolute liver weight in males and hepatocytomegaly in males and females.

The most obvious effects from Cyproconazole in this study were changes in the investigated parameters indicative of liver toxicity. The decreased levels of cholesterol and increased levels of alkaline phosphatase and GGT in high-dose dogs may indicate cholestatic injury. The decreased albumin in high-dose animals may also be due to liver toxicity. Additional evidence of liver toxicity were previously noted increased liver weights and morphologic changes in the liver of mid- and high-dose animals.

~~95~~
88

The LEL in this study, based on adverse effects in liver, was 100 ppm (approximately 4 mg/kg/day). The NOEL was 20 ppm (approximately 0.8 mg/kg/day).

Since the Quality Assurance statement was signed by the Study Director instead of a Quality Assurance Officer, this study is unacceptable for regulatory purposes. Since this study was not inspected by the QAU during the in-life phase, a data audit, signed and dated by a QA Officer, must be submitted to the Agency before this study can be accepted.

Core-classification: supplemental (can be upgraded to minimum provided an acceptable QAU audit is provided as noted above)

CYPROCONAZOLE Tox review 007003

Page 90 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

007003

STUDY TYPE: Teratogenicity Study in Rats TOX. CHEM. NO.: 272E

MRID NO.: 406077-21

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 048712

SPONSOR: Sandoz Corp.

TESTING FACILITY: Research and Consulting Company, A.G., Itingen, Switzerland

TITLE OF REPORT: Teratogenicity Study in Rats with SAN 619F

AUTHOR(S): C. Klotzche

REPORT ISSUED: December 11, 1985

TEST DATES: July 8, 1985 - August 14, 1985

CONCLUSIONS

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Wistar/Han rats (25/group) via oral gavage from day 6 through 15 of gestation at dosage levels of 6, 12, 24 and 48 mg/kg. Evidence of maternal toxicity included inhibited body weight gain during treatment at dosage levels of 12 mg/kg and above and decreased body weight and food consumption among females in the 24 and 48 mg/kg dosage groups. However, since the noted differences in maternal body weights were influenced by treatment-related intrauterine effects (e.g., increased number of resorptions, decreased fetal weight etc.), the evidence for maternal toxicity is equivocal.

Evidence of fetal toxicity was apparent from observed dose-related increases in the number of fetuses with supernumerary ribs at dosages of 6 mg/kg and above. Embryo/fetal toxicity was apparent at 24 and 48 mg/kg from the following observations: decreased total number of fetuses/dam, decreased number of live fetuses/dam, increased percentage and number of fetal resorptions, decreased body weight and incomplete ossification in phalangeal nuclei and the absence of ossification in calcanea.

There was evidence of teratogenicity in the 24 and 48 mg/kg groups. Hydrocephaly was observed in 1 fetus in the 24 mg/kg and 2 fetuses in the 48 mg/kg groups. Cleft palate was observed in 2 fetuses in the 48 mg/kg group.

The NOEL for developmental toxicity was not determined, based on induced fetotoxicity (supernumerary ribs) at 6 mg/kg. The NOEL for maternal toxicity was 6 mg/kg (equivocal).

007003

Test Material

Technical purity: 95.6%; Lot No.: 8507; stereoisomer composition: A=49%, B=51%; description: brownish powder.

Diet

Commercial powdered rat diet (KLIBA No. 21-343-4), provided ad libitum.

Drinking Water

Municipal water in polypropylene bottles, provided ad libitum.

Test Animals

KFM-Wistar albino rats; age at the initiation of treatment: 8 weeks; 26/sex/group.

Acclimation Period

Two weeks

Identification (parent generations)

Cage cards and individual ear marking

Housing

Individually (except during mating period) in Macrolon® polycarbonate cages (size 3), equipped with food and water dispensers; bedding was heat sterilized fine hard wood chips.

Environmental Parameters

Temperature: 23 ± 2°C; relative humidity: 50 ± 20%; light cycle: 12/12; air changes: 15/hr.

METHODS

Group assignment

F_0-generation
Parental animals were randomly allocated to 4 groups.

F_1-generation
Twenty six male and 26 female pups were randomly drawn from each group (1 male and 1 female/litter when possible) for the subsequent part of the study. Brother/sister pairing was avoided.

Dosage groups
Dietary levels of Cyproconazole were 0(control), 4, 20 and 120 ppm.

Diet preparation
Test diet mixtures were prepared weekly from a 1% premix which was prepared at monthly intervals.

92

Stability of test material in the diet
The investigator indicated that data from a separate study showed that the test material was stable in the diet mixture for 14 days. However, these data, which were not included in the report, should be submitted.

Quantitation of test material in the diet
Samples were analysed at the start of the study and at 6 month intervals thereafter. The sampling technique used in these analyses was not described.

Results
Considerable variation was seen in the 4 ppm mixtures (2 analyses showed concentrations at 27 and 45% above nominal), however, since all other measurements approximated the accepted ± 15% of nominal, the increased concentrations at 2 intervals for the low-dose mixture did not compromise the validity of the study (Appended page 1).

Treatment

F_0-generation
These animals were treated continuously for at least 70 days (10 weeks) prior to mating and throughout the mating period. Males were treated for approximately 3 weeks after termination of the mating period and females were treated throughout the gestation, parturition and lactation periods.

F_1-generation
Selected animals were treated from weaning (21st day post partum) for approximately 84 days prior to mating. Treatment continued until necropsy for all animals.

OBSERVATIONS

Parental Animals

Clinical signs
F_0 and F_1: All animals were examined daily for clinical signs of toxicity.

Results
F_0: The investigator indicated that one pregnant high-dose female, which failed to deliver, showed signs of distress such as increased respiration rate and piloerection. No other animal exhibited clinical signs.

F_1: No signs of toxicity were seen in any animal.

Mortalities
F_0 and F_1: A gross necropsy was performed on any animal killed in extremis during the study. Organs/tissues with gross abnormalities were fixed in 4% formalin for possible histologic examination.

Results
F_0: No parental animal died during the study. One mid-dose and one high-dose female were killed because of complete postnatal loss on days 1 and 5, respectively. Also, one pregnant high-dose female that failed to deliver was sacrificed on day 25 post coitum (p.c.).

F_1: No parental animal died during the study. One high-dose female was sacrificed because of complete postnatal litter loss on day 4 post partum (p.p.).

Body weights
F_0: Individual male body weights were recorded at weekly intervals throughout the study. Individual female body weights were recorded weekly during the pre-mating period, on days 0, 7, 14 and 20 p.c. and on days 0, 7, 14 and 21 p.p.

F_1: The selected F_1-animals were individually weighed at weekly intervals until mating. Pregnant and lactating females were recorded on the days indicated for corresponding F_0-females.

Results
F_0 and F_1: No treatment-related differences were evident between males or females and the respective controls during any stage of the study.

Food/compound consumption
F_0 and F_1: Individual food consumption was monitored at the same time as the body weight except during the mating period, when males and females had access to the same feeder.

Results
F_0 and F_1: No treatment-related differences were evident.

Based on food consumption data, the mean compound intake for the respective premating treatment periods are tabulated below:

Cyproconazole Ingestion Levels (mg/kg/day)

Dosage (ppm)	4		20		120	
Generation	Males	Females	Males	Females	Males	Females
F_0	0.28	0.33	1.39	1.67	8.29	9.88
F_1	0.37	0.45	1.77	2.16	10.88	13.30

Mating
F_0: After 10 weeks of dosing each male was paired with a female from the same dose group for a maximum of 21 days (3 weeks at 4 days/week). Vaginal smears were taken daily until sperm was found in the smear. The presence of sperm was recorded and the day of a sperm positive vaginal smear was considered day 0 of pregnancy. The female was removed on day 0 and caged individually.

Females which failed to mate were killed 26 to 28 days after the end of the mating period and subjected to gross and histologic examinations.

F_1: At approximately 15 weeks of age, each female was paired with a male from the same dose group but from a different litter. Vaginal smears were taken as indicated for the F_0-generation.

Results
F_0 and F_1: No treatment-related differences in precoital interval, copulation rate or pregnancy rate were observed in any group.

007003

Parturition

F_0 and F_1: Females were observed at least 3 times/day from day 20 of gestation for signs of parturition. When parturition was observed, the times of onset and completion were recorded and post partum behavior of the female was observed. The day of completion of littering was called day 0 post partum.

Females which failed to deliver by day 25 p.c. or dams whose entire litters were born dead or which died prior to weaning were killed and subjected to necropsy.

Results

F_0: One high-dose pregnant female failed to deliver by day 25 p.c.

The following data indicate that parturition tended to occur later in F_0 treated groups than in corresponding controls (Appended page 2).

Pregnancy Length in F_0 Females

Dose group (ppm)	0	4	20	120
Delivery after day 22 p.c. (%)	7/22 (31.8)	9/23 (39.1)	12/22 (54.5)	11/22 (50.0)
Pregnancy length (days) Mean ± S.D.	22.3 0.5	22.4 0.5	22.6 0.6	22.5 0.5

Although the mean pregnancy length in each treated group exceeded the control value, only the durations shown for the mid- and high-dose groups exceeded the upper-range value from the investigator's historical control data (Appended pages 9 and 10). Therefore, the increased duration of gestation in these groups may be treatment-related.

F_1: Treatment had no apparent effect on parturition or the duration of gestation in F_1 dams.

Necropsy and Histopathology

F_0: The males were killed at approximately 3 weeks after the end of the mating period and the females at the time of the weaning of the F_1 offspring (day 21 p.p.), unless otherwise indicated. These animals were necropsied and each uterus, which was examined for abnormalities and the number of implantation sites, as well as the vagina , cervix, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, coagulating gland, pituitary gland, liver and any tissue/organ with an abnormal lesion were fixed in 4% formalin for possible histologic examination.

The liver of each animal killed at scheduled termination was weighed.

Histologic examinations of noted tissues were performed on all control and high-dose animals as well as on any animal that failed to mate or deliver. Additionally, the liver of all F_0 low- and mid-dose males were examined because of findings in the high-dose group (see below).

F_1: The males were killed approximately 3 weeks after the end of the mating period and the females at the time of weaning of the F_2 offspring (day 21 p.p.). The necropsy was conducted as described for the F_0 animals.

Results

Histopathology

F_0: There was a statistically increase in the incidence of fatty change in the liver of high-dose males, characterized by the investigator as mainly macrovesicular lipid storage in hepatocytes of zones 3 and 2. The severity of this change appeared to be treatment, but not dosage, related.

Fatty Change in the Liver of F_0 Males

Dosage group (ppm)	0	4	20	120
Proportion (%)	10/26 (38.5)	12/26 (46.2)	12/26 (46.2)	19/26* (73.1)
Mean degree of severity	1.6	1.8	1.9	1.8

* $p < 0.05$, Fisher's Exact, Toxicology Branch

Liver weights

Mean absolute and relative liver weights in treated males and females were increased (Appended pages 3, 4, 5 and 6), however, only the increased relative weight in high-dose males was statistically significant ($p<0.05$, Kruskal-Wallis). However, the noted increases in relative weights may be treatment-related at the high-dose in both males and females.

Implantation sites

The mean number of implantation sites was decreased in the high-dose group(7.2% < controls), however, the value was within the historical control range.

Implantation Sites in F_0 Females

Dose group (ppm)	0	4	20	120
Mean	12.5	12.4	12.4	11.6
S.D.	1.6	3.1	1.6	2.5

Historical control range = 9.8-13.0

F_1: There was also a slight (not statistically significant) increase in the incidence of lipid storage in the liver of high-dose males (88% vs. 42% in controls).

All other macroscopic and microscopic changes observed in F_0 and F_1 parental animals appeared to be incidental.

OBSERVATIONS AND PROCEDURES (F_1 and F_2 Generations During Lactation)

Malformations
Gross malformations were noted and recorded. Grossly malformed pups were necropsied.

Results
No treatment-related malformations were evident.

Culling
On day 4 p.p. the size of each litter was adjusted by eliminating extra pups by random selection to yield, if possible, 4 males and 4 females. Litters of less than 8 pups were not altered. Culled pups were subjected to gross necropsy.

Litter size and sex
The litter size and pup sexes were recorded as soon as possible after birth and checked twice at day 0 p.p. and daily thereafter until day 21 p.p.

Results

Litter size
Mean litter sizes were smaller in the high-dose groups at days 0 and 4 p.p. in F_1 pups and at day 4 p.p. in F_2 pups. The mean litter size of the F_1 mid-dose pups was also lower than that of corresponding controls at day 4 p.p. The litter sizes at day 0 p.p. in all groups were within the historical control range; historical control data for litter sizes at day 4 p.p. were not provided in the report. The viability index was decreased in F_1 and F_2 pups and the live birth index was decreased in F_1 pups, all in high-dosage groups.

Litter Sizes at Days 0 and 4 (F_1 and F_2 pups/dam)

Dose group(ppm)		0	4	20	120
Day p.p.	Pups		Mean ; S.D.		
0	F_1	11.2 2.0	11.5 3.2	10.8 3.1	9.8 2.6
	F_2	11.0 2.8	11.7 2.9	12.0 2.2	10.9 2.7
[Historical control = 8.9 - 12.9]					
4	F_1	11.2 2.0	11.3 3.0	10.6 3.2	8.8 3.7
	F_2	10.7 2.7	11.2 2.8	11.8 2.2	10.2 3.5

91

Live Birth, Viability and Lactation Indices (%)

Dose group(ppm)		0	4	20	120
Index	Pups				
Live birth: # alive day 0 x 100 / total # day 0	F_1	97.3 (292/300)	98.9 (264/267)	99.6 (259/260)	95.9 (235/245)
	F_2	94.2 (242/257)	98.8 (257/260)	97.6 (287/294)	98.4 (239/243)
Viability: # alive day 4 x 100 / # alive day 0	F_1	99.7 (291/292)	98.1 (259/264)	98.1 (254/259)	93.2[1/] (219/235)
	F_2	97.5 (236/242)	94.2 (242/257)	98.9 (284/287)	93.3[2/] (223/239)
Lactation: # alive day 21 x 100 / # alive day 4	F_1	100.0 (244/244)	100.0 (176/176)	98.9 (178/180)	99.4 (171/172)
(standardized)	F_2	100.0 (170/170)	98.2 (167/170)	98.4 (185/188)	98.1 (156/159)

1/ One entire litter (11 pups) died.
2/ One entire litter (12 pups) died.

Sex
Treatment did not have an apparent effect on sex distribution in either F_1 or F_2 pups.

Clinical Signs
All pups were examined daily during lactation for clinical signs of toxicity. A necropsy was performed, where possible, on any pups dying or killed in extremis during lactation.

Results
No treatment-related clinical signs were noted.

Noted gross morphologic changes appeared to be incidental: 1 F_1 control pup did not have a tail, irregular skin development (partly hairless at days 9-14 p.p.) was seen in 1 F_1 mid-dose litter and 3 F_2 mid-dose litters.

Body Weights
Pup body weights were recorded by sex soon after birth and at days 4 (before and after culling), 7, 14, and individually on day 21 p.p.

Results
No treatment-related effects were observed at any of the investigated post-natal intervals in either F_1 or F_2 pups.

98

Histopathology
The necropsy procedure for F_1 animals was previously described. F_2 offspring were subjected to the same procedure after weaning.

Results

F_1: There was no evidence that the sporadic lesions observed were associated with treatment.

F_2: Hydronephrosis was a common finding in all groups; the data did not indicate that this or any other finding was induced by treatment.

STATISTICAL ANALYSES

The statistical methods used in this study are described on Appended pages 7 and 8.

DISCUSSION and CONCLUSIONS

Four groups of KFM-Wistar rats were administered technical Cyproconazole at dietary levels of 0(control), 4, 20 and 120 ppm during the pre-mating (10 weeks and 12 weeks, respectively, for the F_0 and F_1 generations), mating, pregnancy and lactation periods to assess the potential reproductive toxicity of the test compound.

Two of the reproductive parameters investigated in parental animals were affected by treatment in F_0 rats only: the duration of gestation at the mid- and high doses was increased and a lower number of implantation sites was seen in high-dose females, both in comparison to respective concurrent control values. Evidence of liver toxicity was seen in high-dose F_0 males (increased lipid storage and relative weight) and females (increased relative weight).

Parameters examined among the offspring which showed treatment-related effects included decreased litter sizes in both the F_1 and F_2 high-dose groups and the F_1 mid-dose group during the early phase of lactation (litters were standardized at day 4 post partum), decreased live birth index in the high-dose F_1 offspring and decreased viability index in the high-dose F_1 and F_2 offspring.

Based on the increased duration of gestation in F_0 dams and the decreased litter sizes observed in F_1 offspring, the LEL in this study was 20 ppm and the NOEL was 4 ppm, which correspond to approximate average dosage levels of 1.7 and 0.4 mg/kg/day, respectively.

Core-classification: minimum (provided test compound stability data and and a description of the sampling technique used for the analyses of dietary levels of test compound are submitted)

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 100 through 102 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007003

The registrant should submit data which show the litter incidence of supernumerary ribs (number of litters/group with the noted change) with appropriate statistical analyses to aid in the determination of a possible NOEL for developmental toxicity in this study.

Core classification - supplementary (can possibly be upgraded to minimum by submitting requested data)

Quality assurance statement: Signed and dated by the QAO

RANGE-FINDING STUDY

The main teratogenicity study was preceded by a pilot study, which was performed to determine appropriate dosage levels of Cyproconazole for the main study. This study was also performed by RCC.

Study Title: Dose-Finding Teratogenicity Study in rats with SAN 619F

Study No.: 048701

Date of report: July 9, 1985

Date of study: May 19, 1985 - June 13, 1985

Author: H. Becker

Materials and Methods

Cyproconazole(Batch No. 8507, purity-95.6%) was administered to mated female Wistar NFM-Han rats (5/group) via gavage at dosage levels of 0(vehicle: distilled water with 4% carboxymethylcellulose sodium salt, 99.5%), 7.5, 30, 75 and 120 mg/kg on days 6 through 15 of gestation. The dams were sacrificed on day 21 of gestation; postmortem examinations included macroscopic inspection of internal organs and uterus (uterine contents and the position of each fetus) as well as corpora lutea counts. The fetuses were sexed, weighed, examined for external gross abnormalities and those without malformations were discarded.

Results

Maternal toxicity was evident from decreased food consumption at 7.5 mg/kg and above and inhibited body weight gain at 30 mg/kg and above, during the treatment period.

Uterine and fetal examinations revealed developmental toxicity at 30, 75 and 120 mg/kg, evident from increased rates of early resorptions (post implantation), decreased fetal body weight and increased incidences of fetuses with cleft palate (especially at 120 mg/kg).

Conclusions

The dosage levels of Cyproconazole chosen for the main teratogenicity study were 6, 12, 24 and 48 mg/kg/day. Based on the treatment-related effects observed in this study, the selected dosages appear to be appropriate.

Core classification: Supplementary data (Range-finding study)

Main Developmental Toxicity Study

TEST MATERIAL

Concentration of technical material = 95.6%; Lot no.: 8507; Code name: SAN 619 F; Isomer composition: not reported; Description: white powder.

TEST ANIMAL DATA

Rats--Wistar/Han; 125 mated females, 25/group; Age at initiation of study = 11 weeks; Body weights at study iniation (post coitum): 180 - 236g; Identification: individual cage numbers and corresponding ear tag.

Group assignment: 25 females were randomly assigned to 1 control and each of 4 dosage groups.

Acclimation: 9 days

Housing: Individually in Makrolon cages with steel mesh tops and standardized granulated softwood bedding.

Food: Pelleted Kliba 343 rat maintenance diet, ad libitum.

Water: municipal tap water: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 22 ± 2°C; Relative humidity - 55 ± 10%; 10-15 air changes/hr; monitored hourly.

METHODS

Mating

Following acclimation, the females were housed with males(1:1) until either the daily vaginal smear was sperm-positive or a copulation plug was observed(designated day 0 post coitum). Each mated female was housed individually.

Preparation of Test Material

The vehicle, distilled water with carboxymethylcellulose sodium salt (CMC; 4%), was added to a weighed amount of Cyproconazole; homogeneity was maintained by constant stirring. Neither the concentration of test compound nor the proportions of water and CMC were indicated.

Administration of Test Material

The test material/vehicle mixtures were prepared daily prior to administration. The test material was administered daily via oral gavage from day 6 through day 15 of gestation. The controls received vehicle only. All groups received a volume of 10 ml/kg body weight with a daily adjustment of individual volume to the actual body weight.

Test Mixture Analysis

Determination of concentration as well as the homogeneity and stability of the test mixtures were performed once during the treatment period. Samples were taken immediately after mixture preparation and again after 90 minutes.

087083

Results

The measured concentrations of Cyproconazole ranged from 83.3 to 108.3% and 83.3 to 116.7% of nominal concentrations immediately after preparation and 90 minutes after preparation, respectively(data on appended page 1).

Maternal Mortality

Animals were observed twice daily for possible mortalities.

Results: None of the females died during the study.

Signs and Symptoms

Animals were examined twice daily.

Results: No apparent treatment-related clinical signs or symptoms were observed.

Number of Dams Examined

Only dams with live fetuses on day 21 post coitum were included in the calculations of body weight gain, corrected body weight gain, mean daily food consumption and reproduction data.

Dose (mg/kg)	0	6	12	24	48
No. of Dams	23	22	23	25	22

Maternal Body Weight and Body Weight Gain

Body weights were measured daily from day 0 until day 21 post coitum. Calculations of body weight gain during the treatment period started on day 6 post coitum (immediately prior to the first administration) and ended on day 16 post coitum (approximately 24 hr after the last administration).

Results: Mean body weights were significantly lower ($p<0.05$, Dunnett's t test) than corresponding control values at 24 mg/kg during gestation days 12 - 21 and at 48 mg/kg during gestation days 8 and 12 - 21 (tabulated on appended pages 2 - 11).

Mean body weight gain during treatment (gestation days 6 - 16) was decreased (>10% lower than control value) in the 12, 24 and 48 mg/kg groups. Inhibited body weight gain was still evident after treatment (days 16-21) in the 24 and 48 mg/kg groups, however, the differences from the control value were less than those noted during the treatment period. Body weight gain before treatment (0-6 days) was comparable between all groups (8.1 - 8.8%).

Mean maternal body weight gain during treatment

Dosage(mg/kg)	0	6	12	24	48
B.W. gain(g)	44	42	39	35	33
% increase	19.6	18.7	17.2	15.7	14.7
Diff. from controls (%)	---	-4.8	-11.4	-20.5	-25.0

106

Mean maternal body weight gain after treatment

Dosage(mg/kg)	0	6	12	24	48
B.W. gain(g)	48	51	53	43	42
% increase	17.8	19.1	19.9	16.7	16.3
Diff. from controls (%)	---	+6.3	+10.4	-10.4	-12.5

Corrected Body Weight Gain

Corrected body weight gain was calculated as follows:

[Body weight on gestation day 21 - Body weight on gestation day 6 - Uterus weight at necropsy on gestation day 21]

Results: Corrected body weight gains were comparable between treated and control groups. Therefore, the noted decreases in body weight and body weight gain among dams in the treatment groups were apparently influenced by increased resorptions and/or decreased fetal weights (see fetal data below).

Mean corrected body weight gain

Dose (mg/kg)	0	6	12	24	48
% gain rel. to day 6	9.5	8.4	7.2	9.3	9.5

Food Consumption

Food consumption was determined on days 6, 11, 16 and 21 of gestation.

Results: Food consumption was decreased in all treated groups during the treatment period; these decreases were statistically significant in the 24 and 48 mg/kg dosage groups.

Mean daily food consumption

Dose (mg/kg)	Gestation Days		
	0 - 6	6 - 16	16 - 21
	[g/day (% diff. from contr.)]		
0	18.7	21.7	23.2
6	18.7(0.0)	20.7(-4.6)	23.4(+0.9)
12	18.8(+0.5)	20.4(-6.0)	23.3(+0.4)
24	18.5(-1.1)	19.4(-10.6)*	23.5(+1.3)
48	18.8(+0.5)	18.7(-13.8)*	24.2(+4.3)

* $p<0.5$, Dunnett's t test

Postmortem Maternal and Fetal Examinations

Maternal examinations included gross macroscopic examination of all internal organs; each uterus was examined for content and fetal position; corpora lutea were counted. Each fetus was removed from the uterus, sexed, weighed and examined for gross external abnormalities.

One third of the live fetuses from each litter were fixed in a mixture of ethanol, formol and acetic acid for subsequent soft tissue examination (technique of Wilson).

The remaining two thirds of live fetuses were cleared in a solution of potassium hydroxide and stained with alizarin red for subsequent skeletal examination (modified technique of Dawson).

The uteri (and contents) of all pregnant females were weighed on the scheduled day of necropsy and used to determine the corrected body weight gain. The uteri of non-pregnant females were placed in an aqueous solution of ammonium sulfide to detect/accentuate possible resorption sites.

Results

Macroscopic Maternal Examination

No treatment-related change was noted in any female.

Uterine Examination (data on appended pages 12 & 13)

Differences between corpora lutea counts, which were comparable between all groups, and implantation site counts showed that the mean number of pre-implantation losses was increased slightly among females in the 48 mg/kg group, however, these losses occurred prior to the scheduled dosage period. Evidence of embryo-/fetal toxicity at the 24 and 48 mg/kg dosage levels was seen as follows (numerical differences relative to mean control values):

1/ Total number of fetuses/dam (alive + dead)decreased*†
2/ Number of live fetuses/dam decreased*
3/ Increased proportion and number* of resorptions
4/ Proportion of post implantation losses increased†

*$p<0.05$, ANOVA based on Wilcoxon ranks

External Fetal Examinations

The following anomalies were observed during this examination:

1/ Runts- 1 fetus in each of the 6, 12, 24 and 48 mg/kg dosages groups

2/ Hydrocephaly†- 1 fetus in the 48 mg/kg group

3/ Cleft palate†- 2 fetuses (2 litters) in the 48 mg/kg group

† Different from historical control incidence(Appended pages 14, 15, 16 & 17)

Sex Ratios

Sex ratios were comparable between all groups.

Fetal Body Weights

Mean fetal body weights were decreased in the 24 and 48 mg/kg groups. Although these weights were only 8.3% below the control value, the differences were statistically significant.

Dosage group mg/kg	0	6	12	24	48
Mean B.W. (g)	4.8	4.8	4.7	4.4*	4.4*
S.D.	0.4	0.4	0.5	0.7	0.7

* $p<0.05$, Analysis of Variance based on Wilcoxon ranks

Soft Tissue Examinations

The following anomalies were observed during this examination:

1/ Hydrocephalus internus† - 1 fetus in the 24 mg/kg and 2 fetuses (2 litters) in the 48 mg/kg groups(both cerebral hemispheres involved in each fetus); hydrocephaly was previously observed in one of these highdose fetuses during the external examination.

2/ Cleft palate† - this anomaly was observed in 2 high-dose fetuses (2 litters) during the external examination and confirmed by this examination.

Skeletal Examinations

Malformations

No treatment-related malformations were observed.

Variations

The only skeletal variations which appeared to be treatment-related were the presence of supernumerary ribs (no. 14 both sides), especially in the 12, 24 and 48 mg/kg groups, and an increased incidence of incompletely ossified phalangeal nuclei and calcanea with still absent ossification, most prominent in the 24 and 48 mg/kg groups(appended pages 18 & 19).

Supernumerary ribs(no. 14)

Dosage group (mg/kg)	0	6	12	24	48
			No. of fetuses (%)		
Left side	3(1.8)	10(5.9)*	17(9.4)**	30(20.5)**	34(29.1)**
Right side	4(2.4)	9(5.3)	14(7.8)*	29(19.9)**	31(26.5)**

* $p<0.05$, ** $p<0.01$, Fisher's exact

† Different from historical control incidence(Appended pages 14, 15, 16 & 17)

Statistical Analyses

The statistical methods used in this study are described on appended page 20.

Discussion and Conclusions

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Wistar/Han rats (25/group) via oral gavage from day 6 through 15 of gestation at dosage levels of 6, 12, 24 and 48 mg/kg.

Evidence of maternal toxicity included inhibited body weight gain during treatment at dosage levels of 12 mg/kg and above and decreased body weight and food consumption among females in the 24 and 48 mg/kg dosage groups. However, since corrected body weight gains were comparable between all groups, the evidence for maternal toxicity is equivocal.

Evidence of fetal toxicity was apparent from observed dose-related increases in the number of fetuses with supernumerary ribs at dosages of 12 mg/kg and above. Embryo/fetal toxicity was apparent at 24 and 48 mg/kg from the following observations: decreased total number of fetuses/dam, increased number of dead fetuses/dam, decreased number of live fetuses/dam, decreased percentage of implantations alive, increased percentage of fetal resorptions, increased percentage of post implantation losses, decreased body weight and incomplete ossification in phalangeal nuclei and the absence of ossification in calcanea. An increase in pre-implanation losses was observed at the 48 mg/kg dose only, prior to the scheduled dosing period. It was indicated in this evaluation that some of these changes were different from the historical control values provided by the test facility, however, the historical data were not presented in a format that would make a comparison of all of the investigated parameters possible. Also, the time frame in which the historical data were generated was not indicated. There was evidence to indicate that embryo/fetal toxicity was induced at the 6 mg/kg level. Although one runt was observed at this dose, the occurrence of 1 runt in each of the other dosage groups with no dose-response relationship probably indicates that this observation was incidental. A slight increase in the number of fetuses with supernumerary ribs was observed at this dose level and the increase was statistically significant on one side.

There was evidence of teratogenicity in the 24 and 48 mg/kg groups. Hydrocephaly was observed in 1 fetus in the 24 mg/kg and 2 fetuses in the 48 mg/kg groups. This malformation was not observed in the pilot study, however, since soft tissue examinations were not performed in that study, hydrocephalus internus would not have been detected. Cleft palate was observed in 2 fetuses in the 48 mg/kg group only. Cleft palate was also seen in the pilot study at dosage levels of 30, 75 and 120 mg/kg.

Although the noted malformations occurred at dosages that induced possible maternal toxicity, the changes indicating toxicity (decreased body weight, body weight gain and food consumption) were not severe and evidence of maternal stress was not remarkable. Also, there was evidence that the noted differences in maternal body weights were influenced by intrauterine effects (e.g., increased resorptions, decreased fetal weights etc.). Therefore, the evidence does not support the conclusion that these malformations are secondary effects from induced maternal toxicity.

The NOEL for developmental toxicity was not clearly apparent in this study since an increased fetal incidence of supernumerary ribs was observed at 6 mg/kg. The litter incidence of this effect could not be determined from the submitted data, therefore, the registrant should submit data showing the litter incidence of supernumerary ribs (number of litters/group with this change) with appropriate statistical analyses to aid in the determination of a possible NOEL for developmental toxicity in this study.

Developmental toxicity NOEL: not attained
Maternal toxicity NOEL: 6 mg/kg (equivocal)

007003

Core classification - supplemental (can possibly be upgraded to minimum by submitting requested data)

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 112 through 131 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel K. Clark Swentzel, 11/18/88 007003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 11/23/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Teratogenicity Study in Rabbits TOX. CHEM. NO.: 272E

MRID NO.: 406077-20

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 053886

SPONSOR: Sandoz Corp.

TESTING FACILITY: Research and Consulting Company, A.G., Itingen, Switzerland

TITLE OF REPORT: Teratogenicity Study in Rabbits with SAN 619F

AUTHOR(S): H. Becker

REPORT ISSUED: March 21, 1986

TEST DATES: October 21, 1985 - December 4, 1985

CONCLUSIONS

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Chinchilla rabbits (16/group) via oral gavage from day 6 through 18 of gestation at dosage levels of 2, 10 and 50 mg/kg.

Evidence of maternal toxicity, which was not remarkable, included inhibited body weight gain during treatment and decreased food consumption during the initial phase of treatment, both at 50 mg/kg. However, since corrected body weight changes between groups were comparable, the evidence of compound-induced maternal toxicity in this study is not convincing.

Embryo/fetal toxicity, observed at 50 mg/kg, was evident from the decreased number of live fetuses/dam and an increased incidence of non-ossification in certain forelimb and hind limb digits. Evidence of embryo/fetal toxicity at dosages of 10 and 50 mg/kg was indicated by an increased incidence of embryonic and fetal resorptions.

Evidence of teratogenicity included hydrocephalus internus, observed in 1 fetus at each dosage level, and agenesia of the left kidney and ureter in 1 high-dose fetus. Hydrocephaly was also seen at 2 dosage levels in a developmental toxicity study in rats with this test material, however, this anomaly did not occur in the control group of either study.

Since a teratogenic response to the test material was observed at the lowest dose tested, a NOEL for developmental toxicity was not attained in this study. Although evidence of maternal toxicity at 50 mg/kg was not remarkable, the 10 mg/kg dosage level is clearly a no-effect level for maternal toxicity.

132

007003

This study is not acceptable for regulatory purposes because: 1) a NOEL for developmental toxicity apparently was not attained and 2) the concentrations of test material were not within the acceptable ± 15% of nominal concentration for the mid- and highdose suspensions immediately after preparation.

Developmental toxicity NOEL: not attained ; < 2 mg/kg/day (LDT)
Maternal toxicity NOEL: 10 mg/kg (equivocal)

Core classification: supplementary

Quality assurance statement: signed and dated by the QAU

RANGE-FINDING STUDY

The main teratogenicity study was preceded by a pilot study, which was performed to determine appropriate dosage levels of Cyproconazole for the main study. This study was also performed by RCC.

Study Title: Dose-Finding Teratogenicity Study in rabbits with SAN 619F

Study No.: 048701

Date of report: October 2, 1985

Date of study: August 12, 1985 - September 10, 1985

Author: H. Becker

Materials and Methods

Cyproconazole(Batch No. 8507, purity-95.6%) was administered to mated female hybrid Chinchilla rabbits (3/group) via gavage at dosage levels of 0(vehicle: distilled water with 4% carboxymethylcellulose sodium salt, 99.5%), 1, 4, 12 and 40 mg/kg on days 6 through 18 of gestation. The dams were sacrificed on day 28 of gestation; postmortem examinations included macroscopic inspection of internal organs and uterus (uterine contents and the position of each fetus) as well as corpora lutea counts. The uteri (and contents) of all pregnant females were weighed to determine corrected body weights. The fetuses were sexed, weighed, examined for external gross abnormalities and prepared for internal examinations which included body cavities (thorax, abdomen, pelvis) and the enclosed organs. The crania were examined for ossification.

Results

Maternal toxicity was evident at 40 mg/kg from decreased food consumption on days 6-11 and 24-28 and a slightly lower mean body weights (7 to 8% below controls) during the treatment period.

Evidence of fetal toxicity at 40 mg/kg included a decrease in the proportion of live fetuses and increases in the proportions of fetal resorptions and post implantation losses. Results of cranial and body cavity examinations were not tabulated, however, the investigator indicated that agenesia of the left or right kidney and ureter was observed in 2 fetuses from 1 litter from the 40 mg/kg group.

Conclusions

Since the noted effects reflecting maternal toxicity at 40 mg/kg were not remarkable, a higher dosage level was selected for the main study. The dosage levels of Cyproconazole chosen for the main teratogenicity study were 2, 10, and 50 mg/kg/day. Based on the treatment-related effects observed in this study, the selected dosages appear to be appropriate.

Core classification: Supplementary data (Range-finding study)

007003

Main Developmental Toxicity Study

TEST MATERIAL

Concentration of technical material = 95.6%; Lot no.: 8507; Code name: SAN 619 F; Isomer composition: not reported; Description: brown-beige powder.

TEST ANIMAL DATA

Rabbits: Chincilla; 64 mated females, 16/group; Age at initiation of study = between 4 & 5 months; Body weights at study iniation (post coitum): 2574-3661g; Identification: individual cage numbers and corresponding number inscribed in ear.

Group assignment: 16 females were randomly assigned to 1 control and each of 3 dosage groups.

Acclimation: 7 days

Housing: Individually in stainless steel cages equiped with automatic cleaning system.

Food: Pelleted Kliba 341 rabbit maintenance diet, ad libitum.

Water: municipal tap water: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 22 ± 3°C; Relative humidity - 40 - 70%; 10-15 air changes/hr; monitored hourly.

METHODS

Mating

Following acclimation, the females were housed with males(1:1) until mating had been observed (designated day 0 post coitum). Each mated female was housed individually.

Preparation of Test Material

The test material/vehicle mixtures were prepared daily prior to administration. The vehicle, distilled water with carboxymethylcellulose sodium salt (CMC; 4%), was added to a weighed amount of Cyproconazole; homogeneity was maintained by constant stirring. The concentration of test compound was not indicated.

Administration of Test Material

The test material was administered daily via oral gavage from day 6 through day 18 of gestation at nominal dosage levels of 2, 10 and 50 mg/kg. The controls received vehicle only. All groups received a volume of 4 ml/kg body weight with a daily adjustment of individual volume to the actual body weight.

Test Mixture Analysis

Determination of concentration as well as the homogeneity and stability of the test mixtures were performed once during the treatment period. Samples were taken immediately after mixture preparation and again after 90 minutes.

Results

The measured concentrations of Cyproconazole ranged from 73.3 to 93.3% and 48.8 to 80.0% of nominal concentrations immediately after preparation and 90 minutes after preparation, respectively(data on appended page 1). The test material concentrations in the dosage suspensions for the 10 and 50 mg/kg groups were not within acceptable limits (± 15% of nominal concentration) immediately after preparation. Ninety minutes after preparation, none of the dosage suspension/test material concentrations were within acceptable limits. Triplicate analyses of each dosage suspension also showed that these mixtures were not homogeneous.

Maternal Mortality

Animals were observed twice daily for possible mortalities.

Results: None of the females died during the study.

Signs and Symptoms

Animals were examined twice daily.

Results: No apparent treatment-related clinical signs or symptoms were observed. One female in the 2 mg/kg group aborted on day 28 p.c. Three dead fetuses (not autolytic) and 3 fetal resorptions were found. The investigator indicated that no anomalies or malformations were found during the external examination of the 3 dead fetuses.

Number of Dams Examined

Only dams with live fetuses on day 21 post coitum were included in the calculations of body weight gain, corrected body weight gain, mean daily food consumption and reproduction data.

Dose (mg/kg)	0	2	10	50
No. of Dams	15	14	16	16

Maternal Body Weight and Body Weight Gain

Body weights were measured daily from day 0 until day 28 post coitum. Calculations of body weight gain during the treatment period started on day 6 post coitum (immediately prior to the first administration) and ended on day 19 post coitum (approximately 24 hr after the last administration).

Results: A comparison of mean body weights between groups did not reveal an obvious treatment-related effect. Initially, the mean weight of the high-dose group was approximately 6% lower than the control value; this deficit was consistent thoughout most of the study but increased slightly to -10.7% on day 28 p.c.

Data for mean group body weight gains (appended page 2) show that the mean weight gain in the high-dose group exceeded that of the controls during the periods before and after treatment (days 0-6 and 19-28) but was lower (-37.6%) during treatment, implicating a possible compound-related effect. Body weight gains in the other treatment groups either exceeded or were comparable to control values at most intervals.

136

007003

Corrected Body Weight Gain

Corrected body weight gain was calculated as follows:

[Body weight on gestation day 28 - Body weight on gestation day 6 - Uterus weight at necropsy on gestation day 28]

Results: Corrected body weight gains were comparable between treated and control groups. Therefore, the noted decreases in body weight and body weight gain among dams in the treatment groups were apparently influenced by increased resorptions (see fetal data below).

Mean corrected body weight gain

Dose (mg/kg)	0	2	10	50
% change rel. to day 6 b.w.	-4.9	-4.5	-3.7	-4.9

Food Consumption

Food consumption was determined on days 6, 11, 15, 19, 24 and 28 of gestation.

Results: Food consumption was decreased (-26.9%, $p<0.05$, Dunnett-test) in the high-dose group during the initial phase of the treatment period (days 6 - 11). Noted differences between treated and control groups at other intervals were not remarkable (appended page 3).

Postmortem Maternal and Fetal Examinations

Maternal examinations included gross macroscopic examination of all internal organs; each uterus was examined for content and fetal position; corpora lutea were counted. Each fetus was removed from the uterus, sexed, weighed and examined for gross external abnormalities and prepared for internal examinations.

The following examinations were performed on each fetus:

1) All fetuses were dissected for body cavity (thorax, abdomen and pelvis) examination; the organs were examined for abnormalities.

2) The skin was removed from the crania of all fetuses in order to examine for ossification.

3) Following fixation in trichloroacetic acid and formaldehyde, the heads were cross-sectioned and the cephalic viscera were examined.

4) The trunk of each fetus was placed in a solution of potassium hydroxide for clearing and stained with alizarin red for subsequent skeletal examination.

Results

Macroscopic Maternal Examination

No treatment-related change was noted in any female.

Uterine Examination (data on Appended pages 4 & 5)

Embryo/fetal toxicity was evident from the following observations:

1/ Total number of fetuses/dam (alive + dead)decreased† - high-dose
2/ Number of live fetuses/dam decreased† - high-dose
3/ Increased proportion and number of resorptions - mid-dose† and high-dose*†

*p<0.05, ANOVA based on Wilcoxon ranks
† Different from historical control incidence(Appended page 6)

External Fetal Examinations

The noted anomalies (thoracogastroschisis, omphalocele and shortened tail) were isolated and had no apparent relationship to treatment.

Sex Ratios

Sex ratios were unrelated to dosage levels of test material.

Fetal Body Weights

Group mean fetal weights were comparable between all groups.

Body Cavity Examinations

The following anomalies were observed during this examination:

1/ Agenesia of the diaphragm- 1 fetus in the control group†† and 1 fetus in the 10 mg/kg group††.

2/ Agenesia of the left-kidney and ureter — 1 fetus in the 50 mg/kg group††.

These isolated findings were not clearly associated with treatment. Agenesia of the left kidney and ureter was noted for 8/3202 fetuses in the historical control data.

Cranial Examination

No abnormalities were noted for any fetus. It was indicated that the stage of ossification was similar between groups.

Cephalic Viscera Examination

Anomalies found:

1/ Microphthalmia- 4 fetuses in one litter in the 10 mg/kg group††.

2/ Hydrocephalus internus- (both hemispheres) 1 fetus in each dosage group††

††Different from historical controls (Appended pages 7,8,9,10,11 & 12)

Skeletal Examinations

Malformations

The observed malformations were isolated, not dosage-related and could not be conclusively associated with treatment(appended page 13).

Variations

An increased incidence of non-ossification of digit no. 5 of the forelimbs and digit no. 4 of the hind limbs was observed among fetuses in the high-dose group (Appended pages 14 & 15). Since this variation was not seen in the controls or the lower dosage groups, it may be associated with treatment.

Statistical Analyses

The statistical methods used in this study are described on appended page 16.

Discussion and Conclusions

Cyproconazole, suspended in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%), was administered daily to pregnant Chinchilla rabbits (16/group) via oral gavage from day 6 throug 18 of gestation at dosage levels of 2, 10 and 50 mg/kg.

Evidence of maternal toxicity, which was not remarkable, included inhibited body weight gain during treatment and decreased food consumption during the initial phase of treatment, both at 50 mg/kg. However, since corrected body weight changes between groups were comparable, the evidence of compound-induced maternal toxicity in this study is not convincing.

Embryo/fetal toxicity, observed at 50 mg/kg, was evident from the decreased number of live fetuses/dam and an increased incidence of non-ossification in certain forelimb and hind limb digits. Evidence of embryo/fetal toxicity at dosages of 10 and 50 mg/kg was indicated by an increased incidence of embryonic and fetal resorptions.

There was evidence of teratogenicity at all dosage levels. Although hydrocephalus internus was seen in only 1 fetus at each dosage level, this anomaly was also observed at 2 dosage levels in a developmental toxicity in rats (Teratogenicity Study in Rats with SAN 619F; MRID No. 406077-21). Furthermore, hydrocephaly was not seen in the control group of either study. The spontaneous incidence of this anomaly among this strain of rat at the test facility was only 9/10,935 (0.08%). Agenesia of the left kidney and ureter was observed in 1 fetus at 50 mg/kg, however, kidney and ureter agenesia was also observed in 2 fetuses (1 litter) at 40 mg/kg in the pilot study. Therefore, this malformation may be treatment-related as well.

Since a possible teratogenic response to the test material was observed at the lowest dose tested, a NOEL for developmental toxicity was not attained in this study. Although evidence of maternal toxicity at 50 mg/kg was not remarkable, the 10 mg/kg dosage level is clearly a no-effect level for maternal toxicity.

This study is not acceptable for regulatory purposes because: 1) a NOEL for developmental toxicity apparently was not attained and 2) the concentrations of test material were not within the acceptable ± 15% of nominal concentration for the mid- and highdose suspensions immediately after preparation.

007003

Developmental toxicity NOEL: not attained; <2 mg/kg/day (LDT)
Maternal toxicity NOEL: 10 mg/kg (equivocal)

Core classification: supplementary

140

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 141 through 156 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel K. Clark Swentzel 12/2/88 007003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 12/7/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Two-Generation Reproduction in Rats TOX. CHEM. NO.: 272E

MRID NO.: 406077-23

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
SYNONYMS: Cyproconazole; SAN 619F

something's missing ?

STUDY NUMBER(S): 6712/87

SPONSOR: Sandoz Corp.

TESTING FACILITY: Sandoz Ltd., Agrotoxicology, Basel, Switzerland

TITLE OF REPORT: San 619F, 2-Generation Study in Rats

AUTHOR(S): B. Eschbach, R. Aerni, J.C. Karapally, R. Bourry

REPORT ISSUED: July 8, 1987

TEST DATES: February 27, 1986 - December 1986

CONCLUSIONS

Four groups of KFM-Wistar rats were administered technical Cyproconazole at dietary levels of 0(control), 4, 20 and 120 ppm during the pre-mating (10 weeks and 12 weeks, respectively, for the F_0 and F_1 generations), mating, pregnancy and lactation periods to assess the potential reproductive toxicity of the test compound.

Two of the reproductive parameters investigated in parental animals were affected by treatment in F_0 rats only: the duration of gestation at the mid- and high doses was increased and a lower number of implantation sites was seen in high-dose females, both in comparison to respective concurrent control values. Evidence of liver toxicity was seen in high-dose F_0 males (increased lipid storage and relative weight) and females (increased relative weight).

Parameters examined among the offspring which showed treatment-related effects included decreased litter sizes in both the F_1 and F_2 high-dose groups and the F_1 mid-dose group during the early phase of lactation (litters were standardized at day 4 post partum), decreased live birth index in the high-dose F_1 offspring and decreased viability index in the high-dose F_1 and F_2 offspring.

Based on the increased duration of gestation in F_0 dams and the decreased litter sizes observed in F_1 offspring, the LEL in this study was 20 ppm and the NOEL was 4 ppm, which correspond to approximate average dosage levels of 1.7 and 0.4 mg/kg/day, respectively.

Core-classification: minimum (provided test compound stability data and and a description of the sampling technique used for the analyses of dietary levels of test compound are submitted)

Quality assurance statement: signed and dated by the QAU

151

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 158 through 164 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007003

Reviewed by: Byron T. Backus Byron T. Backus 12/2/88
Section 2, HFASB (TS-769C)

Secondary Reviewer: John H. S. Chen, D.V.M. John H S Chen 12/2/88
Section 1, HFASB (TS-769C)

Tertiary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/6/88
Section 2, HFASB (TS-769C)

DATA EVALUATION REPORT I

STUDY TYPE: Gene Mutation - Ames Salmonella microsome reverse mutation assay TOX CHEM NO. 272E

ACCESSION NUMBER: 406077-25 MRID NO:

TEST MATERIAL: SAN 619 F, 95.6%

SYNONYMS: SAN 619 F

STUDY NUMBER(S): E-9528

SPONSOR: Sandoz Crop Protection Corporation

TESTING FACILITY: Hazelton Biotechnologies
Landjuweel 11
3905 De Veenendaal
The Netherlands

TITLE OF REPORT: Mutagenicity Evaluation of SAN 619F in the Ames Salmonella Microsome Reverse Mutation Assay

AUTHOR(S): Hoorn, A.J.W.

REPORT ISSUED: 09/19/86

CLASSIFICATION: Acceptable with comments

CONCLUSIONS:

1. No evidence was observed of a mutagenic effect at the histidine locus in any of the Salmonella typhiumurium strains (TA-98, TA-100, TA-1535, TA-1537 and TA-1538) at dose levels of 1, 5, 10, 100, 500 or 1000 ug/plate of the test material either with or without rat S9 mix. There was complete cytotoxicity (as evidenced by a lack of revertant colonies) at 2500 ug/plate and above.

2. Considering that the initial rangefinding assay showed very little evidence for cytotoxicity at 1250 ug/plate, it would probably have been more appropriate to use this dose level,

rather than 1000 ug/plate, in the subsequent mutagenicity assays. However, it is concluded that the difference between 1000 and 1250 ug/plate is not that great, and the study and its findings are acceptable. It is noted that there is apparently a fairly steep dose response for cytotoxicity occurring between 1000 and 2500 ug/plate.

A. MATERIALS:

1. Test compound: SAN 619 F, Description: a brown powder. Batch no. 8507. Purity 95.6%. Material used was received July 11, 1986. DMSO was the solvent.

2. Positive control compounds: (without activation) sodium azide at 10 ug/plate for TA 1535 and TA 100; 2-nitrofluorene at 10 ug/plate for TA 1538 and TA 98; quinacrine mustard at 5 ug/plate for TA 1537; (with activation) 2-anthramine at 2.5 ug/plate for all strains.

3. Test microorganisms: the following Salmonella typhimurium strains, all with mutations in the histidine operon: TA 1535 and TA 100 (base-pair substitution); and TA 1537, TA 1538 and TA 98 (frameshift). All these strains also have a defective lipopolysaccharide coat (making them more permeable to large molecules), and a defect in the repair of ultraviolet-induced DNA damage. TA 98 and TA 100 carry a transfer factor plasmid conferring resistance to ampicillin and also causing an increase in errror-prone DNA repair.

   These strains were originally obtained from Dr. Bruce Ames. They were maintained at 4° on complete medium plates supplemented with ampicillin (25 ug/ml) for TA 98 and TA 100 for selection for maintenance of the plasmid.

4. S9: this was a commercial preparation derived from the livers of Aroclor 1254-treated male Sprague-Dawley rats. One ml of S9 mix contained the following components: S9, 100 uliters; NADP, 4 umoles, D-glucose-6-phosphate, 5 umoles; KCl, 33 umoles; sodium phosphate buffer, pH 7.4, 100 umoles.

B. STUDY DESIGN:

1. Dose rangefinding assay:

"Doses were selected...based on a preliminary dose range-finding test with the strain TA-100." Doses ranged from 1.22 to 10,000 ug/plate.

"To a sterile test tube containing 2.0 ml of overlay agar (placed in a 43°-45°C water bath)...were added:

0.05 - 0.10 ml of a solution of the test material to give the appropriate dose.

0.1 to 0.2 ml of indicator organism.

0.5 ml of 0.2M phosphate buffer, pH 7.4."

"This mixture was swirled gently and then poured onto minimal agar plates... After the top agar had set, the plates were incubated at about 37°C for approximately two days. The number of his+ revertant colonies growing on the plates were counted and recorded."

"A reduction in the number of revertants, appearance of microcolonies, or clearing of the background lawn on the test material treated plates as compared to the solvent control plates were considered as indications of toxicity by the test material."

2. Mutagenicity assay:

Doses (1.0 to 5,000 ug per plate in the first run; 1.0 to 2,500 ug per plate in the second run), selected on the basis of the preliminary rangefinding assay, were used.

Nonactivation:

The following were combined at 43-45°C:

(a) 2 ml of molten overlay agar.

(b) 0.05 ml of a solution of the test chemical to yield the appropriate dose.

(c) 0.2 ml of indicator organism.

(d) 0.5 ml of 0.2M phosphate buffer, pH 7.4.

"The mixture was swirled gently and then poured onto minimal agar plates... After the top agar had set, the plates were incubated at about 37°C for approximately two days. The number of his+ revertant colonies growing on the plates were counted and recorded."

Activation:

"The activation assay was run concurrently with the nonactivation assay. The only difference was the addition of 0.5 ml of S9 mix...in place of 0.5 ml of phosphate buffer that was added in nonactivation assays."

3. Evaluation:

"Statistical methods are not currently used, and evaluation is based on the criteria included in this protocol." From p. 36:

"Most data sets are evaluated using the following criteria:

(a) Strains TA-1535, TA-1537 and TA-1538

If the solvent control is within the normal range, a test material producing a positive response equal to at least three times the solvent control value is considered mutagenic.

(b) Strains TA-98 and TA-100

If the solvent control is within the normal range, a test material producing a positive response equal to at least twice the solvent control value for TA-98 and TA-100 is considered mutagenic.

The following normal range of revertants for solvent controls are generally considered acceptable:

TA-1535:	8-30
TA-1537:	4-30
TA-1538:	10-35
TA-98:	20-75
TA-100:	80-250

(c) Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain...and because TA-1538 and TA-98 are both derived from the same parental strain...to some extent there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen..."

(d) A dose-related increase in mutagenic response is also usually present for a positive test substance.

4. A Good Laboratory Practice statement is provided on p. 3 of the report, and a quality assurance statement on p. 29.

C. RESULTS:

1. Rangefinding assay:

The following findings are among those presented in table 1, p. 20:

Test compound ug/plate	Number of colonies/plate	Appearance of background lawn
Solvent control*	107 & 112	Normal
312.5	117	Normal
625.0	120	Normal
1250.0	85	Normal
2500.0	25	Microcolonies
5000.0	0	Clear
10000.0	0	Clear

2. Mutagenicity assay:

Trial I - without activation (from tables 2-3, p. 21-22):
Representative findings:

3 plates/dose level	Mean revertants/plate				
	TA-1535	TA-1537	TA-1538	TA-98	TA-100
Solvent control*	10.0	9.3	10.7	20.0	112.7
Positive control**	1154.7	1169.7	1568.0	1262.7	1080.7
1 ug SAN 619 F	13.7	8.3	16.7	18.0	137.0
500 ug SAN 619 F	14.3	4.3	10.0	22.0	144.7
1000 ug SAN 619 F	11.3	8.0	12.7	20.0	125.7

At 2500 and 5000 ug SAN 619F no revertants were observed

* 50 ul solvent (DMSO) per plate
**TA-1535 and TA-100: 10 ug/plate sodium azide; TA-1537: 5 ug /plate Quinacrine mustard; TA-1538 and TA-98: 10 ug/plate 2-nitrofluorene.

Trial I - with activation (from tables 4-5, p. 23-24):
Representative findings:

3 plates/dose level	Mean revertants/plate				
	TA-1535	TA-1537	TA-1538	TA-98	TA-100
Solvent control*	9.3	8.7	24.0	31.3	107.3
Positive control**	658.0	504.7	2229.3	2138.7	2251.0
1 ug SAN 619 F	9.0	9.3	24.0	31.0	128.0
500 ug SAN 619 F	6.7	10.0	21.3	33.3	131.7
1000 ug SAN 619 F	8.3	9.3	19.7	32.7	127.0

At 2500 and 5000 ug SAN 619F no revertants were observed

* 50 ul solvent (DMSO) per plate
**2.5 ug/plate 2-anthramine (all cultures)

Trial II - without activation (from tables 6-7, p. 25-26):
Representative findings:

3 plates/dose level	Mean revertants/plate				
	TA-1535	TA-1537	TA-1538	TA-98	TA-100
Solvent control*	10.7	15.7	13.3	26.3	99.3
Positive control**	1186.3	1334.7	1845.3	1496.7	1158.0
1 ug SAN 619 F	14.0	15.7	14.3	22.7	123.3
500 ug SAN 619 F	14.3	8.3	13.0	34.7	109.3
1000 ug SAN 619 F	10.0	13.7	10.3	27.3	99.0
At 2500 ug SAN 619F no revertants were observed					

* 50 ul solvent (DMSO) per plate
**TA-1535 and TA-100: 10 ug/plate sodium azide; TA-1537: 5 ug /plate Quinacrine mustard; TA-1538 and TA-98: 10 ug/plate 2-nitrofluorene.

Trial II - with activation (from tables 8-9, p. 27-28):
Representative findings:

3 plates/dose level	Mean revertants/plate				
	TA-1535	TA-1537	TA-1538	TA-98	TA-100
Solvent control*	9.7	8.3	25.3	39.0	100.7
Positive control**	557.0	565.0	2266.0	2122.3	2044.3
1 ug SAN 619 F	11.7	9.0	24.7	30.3	95.7
500 ug SAN 619 F	7.7	6.0	21.7	32.0	117.0
1000 ug SAN 619 F	6.7	8.0	19.0	33.7	106.7
At 2500 ug SAN 619F no revertants were observed					

* 50 ul solvent (DMSO) per plate
**2.5 ug/plate 2-anthramine (all cultures)

D. DISCUSSION:

No evidence of a dose-related increase in mutation frequency at the histidine locus was observed under the conditions of this assay (both with and without rat S9 activation) in any of the Salmonella typhimurium strains used. The study was conducted in replicate, with 3 plates/strain/dose level on each occasion. The results obtained from the strain specific control compounds and the positive control compound to ensure the efficacy of the activation system demonstrated the sensitivity of the assay system with or without metabolic activation for this study.

There is very little indication that the test material was assayed within the range associated with substantial (50-90%) cytotoxicity for any of the S. typhimurium strains used. Only in the preliminary rangefinding assay was there a substantial reduction in the number of revertant colonies/plate at 2500 ug/plate, with possibly some (about 25-30%) reduction in revertants at 1250 ug/plate. In the subsequent mutagenic assays the subject chemical was tested at 1000 and 2500 ug/plate, with no intervening dose levels. For all of the strains there was no evidence of cytotoxicity at 1000 ug/plate, but

apparently complete cytotoxicity (no revertant colonies were present) occurred at 2500 ug/plate. Considering that the initial rangefinding assay showed only limited evidence for cytotoxicity at 1250 ug/plate, it would probably have been appropriate to use this dose level, rather than 1000 ug/plate, in the subsequent mutagenicity assays. It is concluded, however, that this is a minor flaw, and the study and its conclusions are acceptable. The findings can be interpreted as indicating a fairly steep dose response for cytotoxicity occurs between 1000 and 2500 ug/plate.

Reviewed by: Byron T. Backus Byron T. Backus 12/02/88
Section 2, HFASB (TS-769C)

Secondary Reviewer: John H. S. Chen, D.V.M. John H. S. Chen 12/2/88
Section 1, HFASB (TS-769C)

Tertiary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/6/88
Section 2, HFASB (TS-769C)

DATA EVALUATION REPORT III

STUDY TYPE: In vitro HGPRT gene mutation test with CHO Cells TOX CHEM NO. 272E

ACCESSION NUMBER: 406077-26 MRID NO:

TEST MATERIAL: SAN 619 F, 94.4%

SYNONYMS: SAN 619 F

STUDY NUMBER(S): LMP 099A

SPONSOR: Sandoz AG

TESTING FACILITY: Laboratorium fur Mutagenitasprunfungr
Technische Hochschule
Darmstadt, Germany

TITLE OF REPORT: SAN 619 F: In vitro HGPRT Gene Mutation Test using Chinese Hamster Ovary Cell Line V79

AUTHOR(S): Miltenburger, H.G.

REPORT ISSUED: 02/28/85

CLASSIFICATION: Acceptable

CONCLUSIONS:

1. There was no indication of mutagenic activity either with or without S9 activation at any of the dose levels (0, 20, 50, 100 or 200 ug/ml). The test material was soluble only up to 200 ug/ml, at which there was little or no evidence of cytotoxicity. The experiment was done twice. The positive controls (without S9: EMS at 1.0 mg/ml; with S9: DMBA at 15.4 ug/ml) gave appropriate responses.

2. The study is acceptable.

A. MATERIALS:

1. Test compound: SAN 619 F, purity 94.4%. Supplied from SANDOZ AG. Dissolved in DMSO ("solution prepared on day of experiment"), with "a final concentration in culture medium" of 1%.

2. Positive control materials: EMS (Ethylmethanesulfonate) from Merck-Schuchardt, batch no. 2188 593 (used without S9 metabolic activation at 1 mg/ml) and 9,10-dimethyl-1,2-benzanthracene (DMBA) from Sigma Chemie GmbH (15.4 ug/ml with S9 metabolic activation).

3. Indicator cells: Chinese hamster cell line V79. Used because of the high proliferation rate (doubling time 12-16 hr in stock cultures) and high plating efficiency (70-90%) of unplated cells. "The cells have a stable karyotype with a modal chromosome number of 22 $\pm$ 1."

4. S9: "obtained from the liver of 8-12 weeks old male Wistar rats, strain CFHB (weight ca. 150-200 g) which had been given a single i.p. injection of 500 mg/kg b.w. Aroclor 1254...in olive oil 5 days previously." The S9 had been frozen and stored in liquid nitrogen. The S9/cofactor mixture consisted of 8mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 5 mM NADP, 100 mM Na_2HPO_4 (pH 7.4) and 0.3 mg/ml of S9.

B. STUDY DESIGN:

1. Cell exposure: "Two days old logarithmically growing stock cultures more than 50% confluent were trypsinized and a single cell suspension was prepared...After 24 h the medium was replaced with medium containing 2% FCS (= fetal calf serum) and the test substance, without S9 mix and with 20 ul/ml S9 mix. After 4 h this medium was replaced with normal medium after rinsing..."

2. Rangefinding cytotoxicity assay: From p. 7: "The test substance could be dissolved up to precipitation which occurred in the cell culture medium at concentrations of more than 200 ug/ml." The SAN 619 F was therefore tested for cytotoxicity at 0, 20, 50, 100 and 200 ug/ml. There was no conclusive evidence for cytotoxicity at 200 ug/ml, as absolute plating efficiences (p. 20 of the report) were in the range of 85-90% both with and without S9, while relative plating efficiencies were 87.6% (without S9) and 90.6% (with S9).

3. Mutagenicity assay: The same concentrations used in the rangefinding were used in the mutagenicity assay, both with and without S9 mix. The following procedure was used (from p. 17):

   Day 1: Subculturing of a log-phase culture
   a) About 400 cells in 5 ml medium/25 cm^2 plastic flasks for plating efficiency; in duplicate per experimental point.
   b) 1 x 10^6 cells in 30 ml medium/175 cm^2 plastic flask; for the mutagenicity test, 1 flask per experimental point.

   Day 2: Treatment of a) and b)

   Day 5: Subculturing of b) in 175 cm^2 plastic flasks.

   Day 8: Fixation and staining of colonies of a)

   Day 9: Subculturing of b) in five 80 cm^2 plastic flasks containing selective medium (containing 11 ug/ml thioguanine); mutant selection and subculturing of b) in two 25 cm^2 flasks for plating efficiency.

   Day 16: Fixation and staining of colonies in b)-derived flasks seeded on day 9.

   Incubation was at 37° C; staining was with 10% methylene blue in 0.01n KOH.

4. Evaluation of results:

   "The test substance is classified as mutagenic if it induces for at least one of the test substance concentrations reproducibly a mutation frequency that is three times higher than the spontaneous mutation frequency in this experiment."

   "The test substance is classified as mutagenic if there is a reproducible concentration-related increase in the mutant frequency."

5. A signed statement of compliance with good laboratory practice is provided on p. 3, and an additional statement of compliance, along with a quality assurance statement, is on page 9.

C. RESULTS:

1. Experiment Run I:

Preliminary cytotoxicity:

Without activation (from Table I, p. 20):

Treatment	Mean no. colonies per plate	Plating Efficiency % absolute	Plating Efficiency % relative
Negative control	522	105.5	100.0
Negative control with solvent	484	97.8	100.0
Positive control - EMS 1 mg	454	91.7	87.0
Test substance - 20 ug	500	101.0	103.3
Test substance - 50 ug	470.5	95.1	97.2
Test substance - 100 ug	471	95.2	97.3
Test substance - 200 ug	424	85.7	87.6

With S9 activation (from Table I, p. 20):

Treatment	Mean no. colonies per plate	Plating Efficiency % absolute	Plating Efficiency % relative
Negative control	480	97.0	100.0
Negative control with solvent	482	97.4	100.0
Positive control-DMBA 15.4 ug	274.5	55.5	57.0
Test substance - 20 ug	455.5	92.0	94.5
Test substance - 50 ug	445.5	90.0	92.4
Test substance - 100 ug	473	95.6	98.1
Test substance - 200 ug	436.5	88.2	90.6

CHO/HGPRT assay:
Without S9 activation (from Table III, p. 22):

Treatment	Mean no. mutant colonies per plate ± S.D.	Mutant colonies per 10^6 cells
Negative control	13.0 ± 5.3	26.3
Negative control (solvent)	28.8 ± 4.7	70.7
Positive control-EMS 1 mg	194.3 ± 9.6	428.6
Test substance - 20 ug	19.2 ± 4.5	49.8
Test substance - 50 ug	21.4 ± 4.0	45.2
Test substance - 100 ug	27.0 ± 6.7	48.8
Test substance - 200 ug	12.2 ± 2.3	24.0

With S9 activation (from Table III, p. 22):

Treatment	Mean no. mutant colonies per plate ± S.D.	Mutant colonies per 10^6 cells
Negative control	9.2 ± 2.8	28.4
Negative control (solvent)	7.6 ± 3.6	27.9
Pos. control DMBA 15.4 ug	52.4 ± 9.5	154.8
Test substance - 20 ug	9.0 ± 4.5	23.2
Test substance - 50 ug	18.0 ± 3.7	38.2
Test substance - 100 ug	14.0 ± 5.4	30.7
Test substance - 200 ug	13.8 ± 4.3	32.2

2. Experiment run 2:

Preliminary cytotoxicity:

Without S9 activation (from Table IV, p. 23):

Treatment	Mean no. colonies per plate	Plating Efficiency % absolute	Plating Efficiency % relative
Negative control	458.5	92.4	100.0
Negative control with solvent	500.5	100.9	100.0
Positive control - EMS 1 mg	442.5	89.2	96.5
Test substance - 20 ug	471.5	95.1	94.2
Test substance - 50 ug	520	104.8	103.9
Test substance - 100 ug	498	100.4	99.5
Test substance - 200 ug	413	83.3	82.5

With S9 activation (from Table IV, p. 23):

Treatment	Mean no. colonies per plate	Plating Efficiency % absolute	Plating Efficiency % relative
Negative control	465	93.8	100.0
Negative control with solvent	449.5	90.6	100.0
Positive control-DMBA 15.4 ug	122	24.6	27.1
Test substance - 20 ug	478	96.4	106.3
Test substance - 50 ug	465	93.8	103.4
Test substance - 100 ug	465.5	93.9	103.6
Test substance - 200 ug	371.5	74.9	82.6

CHO/HGPRT assay:

Without S9 activation (from Table VI, p. 25):

Treatment	Mean no. mutant colonies per plate + S.D.	Mutant colonies per 10^6 cells
Negative control	11.0 ± 3.8	35.3
Negative control (solvent)	7.2 ± 3.1	24.7
Positive control-EMS 1 mg	112.8 ± 10.6	394.2
Test substance - 20 ug	16.2 ± 4.4	54.4
Test substance - 50 ug	12.2 ± 3.6	38.2
Test substance - 100 ug	9.4 ± 2.9	23.7
Test substance - 200 ug	13.0 ± 2.0	39.2

With S9 activation (from Table VI, p. 25):

Treatment	Mean no. mutant colonies per plate + S.D.	Mutant colonies per 10^6 cells
Negative control	8.2 ± 3.0	24.6
Negative control (solvent)	14.6 ± 2.3	43.8
Pos. control DMBA 15.4 ug	49.2 ± 3.3	93.5
Test substance - 20 ug	11.4 ± 3.5	31.7
Test substance - 50 ug	9.4 ± 2.2	21.3
Test substance - 100 ug	9.6 ± 4.3	29.4
Test substance - 200 ug	12.4 ± 3.8	27.7

D. DISCUSSION:

There was no indication of mutagenic activity either with or without S9 activation. The material was tested in two separate experiments. The positive control materials gave an appropriate response. There was little or no evidence for cytotoxicity at the highest dose level (200 ug/ml) used in both of these experiments, but this concentration was at the limit of solubility for the test compound.

It is concluded that the study and its findings are acceptable.

Reviewed by: Byron T. Backus Byron T. Backus 12/01/88
Section 2, HFASB (TS-769C)

Secondary Reviewer: John H. S. Chen, D.V.M. John H S Chen 12/5/88
Section 1, HFASB (TS-769C)

Tertiary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/14/88
Section 2, HFASB (TS-769C)

DATA EVALUATION REPORT II

STUDY TYPE: Unscheduled DNA Synthesis (UDS) in vitro in rat hepatocytes TOX CHEM NO. 272E

ACCESSION NUMBER: 406077-27 MRID NO:

TEST MATERIAL: SAN 619 F, 94.4%

SYNONYMS: SAN 619 F

STUDY NUMBER(S): LMP 099B

SPONSOR: Sandoz AG

TESTING FACILITY: Laboratory for Mutagenicity Testing of the Technical University Darmstadt (LMP DARMSTADT)

TITLE OF REPORT: SAN 619 F: Unscheduled DNA Synthesis (UDS) Test in vitro in Rat Hepatocytes

AUTHOR(S): Miltenburger, H.G.

REPORT ISSUED: 02/28/85

CLASSIFICATION: Not Acceptable

CONCLUSIONS:

1. There was no indication of an increased level of incorporation of ^{3}H-TdR as a result of exposure to SAN 619 F, either as an isolated occurrence at a single dose level or as part of a dose-related trend. The dose levels used were 0.25, 3.3, 6.6, 10 and 25 ug/ml. The study was conducted with 6 replicate cultures/dose level, and the positive control elicited an appropriate response.

2. The highest dose level should probably have been greater than 25 ug/ml, particularly as no indication of a decrease in incorporation of ^{3}H-TdR (either with respect to the negative control or lower dose levels) occurred. The trypan-blue test

showed complete cytotoxicity at 100 ug/ml. From this it is concluded that the material should have been tested at 50 ug/ml. An additional consideration is that the spontaneous negative control values should have been in the range between 50 and 150 dpm/ug DNA. Since the negative control values from this study were higher than this value (208.9 dpm/ug DNA) there is some question as to whether the assay system was conducted under the optimal conditions to detect DNA repair.

A. MATERIALS:

1. Test compound: SAN 619 F, purity 94.4%. Supplied from SANDOZ AG. Dissolved in DMSO ("solution prepared on day of experiment"), with "a final concentration in the nutrient medium" of 1%.

2. Positive control material: 7,12-dimethylbenz(a)anthracene (DMBA) from Sigma Chemie GmbH.

3. Indicator cells: hepatocytes from 8-12 week old male Wistar CF HB rats (150-200 g).

B. STUDY DESIGN:

1. Rat hepatocytes were obtained by perfusing the liver with a collagenase-containing medium through the vena portae. "The hepatocytes were removed from the liver and washed twice with the perfusion solution without collagenase. The crude cell suspension was filtered through a 40 um stainless steel mesh to yield a single cell suspension. For the viability test the cells were stained with trypan blue."

2. Dose selection: From p. 14: "The toxicity of the test substance was determined in a preexperiment by measuring the dose related incorporation of radioactive thymidine up to the limit of solubility of the test substance. According to these data the dose range was chosen."

3. UDS assay: Five concentrations (0.25, 3.3, 6.6, 10 and 25 ug/ml) of the SAN 619 F were tested. "Aliquots containing 3.5 - 4 x 10^6 hepatocytes were transferred into 4 ml of culture medium supplemented with hydroxyurea (15 mM) in 25 ml erlenmeyer flasks. The flasks were agitated for one hour in an orbital water bath (37° C) set at 100 oscillations per minute. Then the test substance together with (^{3}H)-TdR (0.7 uCi/ml) was added and the incubation at 37° C was continued for another 3 hours."

After 4 hours' incubation the flasks were transferred to an ice bath. The cells were washed twice and 0.5 mg/ml thymi dine was added to each sample. The nuclei were isolated by lysing cells in 2 ml of a pH 8.5 solution containing 10 mM Tris-HCl; 15 mM NaCl; 1.5 mM $MgCl_2$; and Nonidet P40 0.5%. After 10 minutes the nuclei were spun down; "the pellet was washed twice with the lysing solution but without Nonidet P 40."

The nuclei were lysed for 30 minutes in 2.5 ml of a pH 10 solution containing 2.5 mM EDTA; 2% SDS; 0.1 M glycine; and 1 mg/ml proteinase K. The DNA was precipitated with 10% trichloracetic acid, kept at 4°C overnight, then was spun down and the pellet was redissolved in 1 ml 5% trichloro-acetic acid.

0.2 ml aliquots were used for liquid scintillation counting while other 0.2 ml aliquots were used for the determination of DNA content.

The values obtained (ug of DNA in 0.2 ml and disintegrations per minute - dpm - in 0.2 ml) were used to calculate the dpm/ug DNA.

4. Assay evaluation criteria:

"A concentration-related increase of the (^{3}H)-TdR incorporation or a significant increase after treatment with a least one concentration above that of the negative control is regarded as a positive response."

"A test substance is regarded as negative, when the (^{3}H)-TdR incorporation is indistinguishable from the negative control in all groups treated with the test substance up to cytotoxic levels."

5. A signed Quality Assurance Statement is provided on p. 8, along with dates of inspection.

C. RESULTS:

1. Preliminary assay for cytotoxicity: The following values for viability according to the trypan-blue test are reported on p. 17:

Dose	% Viability
0 ug/ml	100
5 ug/ml	104
25 ug/ml	73
50 ug/ml	44
100 ug/ml	0

2. ^{3}H TdR incorporation:

See the appended sheet from p. 19 of the report.

D. DISCUSSION:

There was no indication that there was an increased level of incorporation of ^{3}H-TdR as a result of exposure to the SAN 619 F, either as an isolated occurrence at the highest dose level, or as part of a dose-related trend. The study was conducted with 6 replicate cultures/dose level, and the positive control elicited an appropriate response.

It is noted that the highest dose level should probably have been greater than 25 ug/ml, as there was 73% viability with respect to controls in a preliminary trypan-blue study at this dose level, and there was 44% viability at 50 ug/ml. At 25 ug/ml in the mutagenicity assay there was no indication of a decrease in incorporation of ^{3}H-TdR at 25 ug/ml with respect to the negative control (mean values of 212.4 and 208.9 dpm/ug DNA respectively). The relatively consistent response at each of the dose levels (and the fact that this was essentially the same response as for the negative control) indicates there was no dose response relationship. The trypan-blue test showed complete cytotoxicity at 100 ug/ml. From this it is concluded that 50 ug/ml might have been an acceptable maximum dose, but that even if the material had been tested at this level, it is unlikely that results indicating a mutagenic response would have been obtained.

However, an additional consideration is that according to the acceptable protocol for liquid scintillation counting (LSC) of UDS in rat hepatocytes recommended by EPA (Unscheduled DNA Synthesis Tests: A report of the U.S. Environmental Protection Agency Gene-Tox Program, Ann D. Mitchell et al., January 26, 1983) historical values for the spontaneous background frequencies of ^{3}H-TdR incorporation in negative control LSC-UDS samples should be in a range between 50 and 150 dpm/ug DNA. Since the negative control values obtained in this study (208.9 dpm/ug DNA) are not within this acceptable range, there is a question as to whether the assay system was conducted under the optimal conditions to detect a DNA-damaging effect. The study is therefore classified as not acceptable.

Reviewed by: Byron T. Backus Byron T. Backus 12/01/88
Section 2, HFASB (TS-769C)

Secondary Reviewer: John Chen, D.V.M. John H Chen 12/1/88
Section 1, HFASB (TS-769C)

Tertiary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/6/88
Section 2, HFASB (TS-769C)

DATA EVALUATION REPORT V

STUDY TYPE: Mutagenicity - micronucleus test (in vivo mouse) TOX CHEM NO.

ACCESSION NUMBER: 406077-28 MRID NO:

TEST MATERIAL: SAN 619F

SYNONYMS:

STUDY NUMBER(S): Laboratory Project ID 249.215.005

SPONSOR: Sandoz

TESTING FACILITY: Litton Bionetics
Landjuweel 11
3905 De Veenendaal
The Netherlands

TITLE OF REPORT: Mutagenicity Evaluation of SAN 619 F in the in vivo Mouse Micronucleus Assay

AUTHOR(S): Taalman, R.D.F.M.

REPORT ISSUED: 01/10/85
12/19/84 (study completion date)

CLASSIFICATION: Not acceptable (additional information needed as noted below)

CONCLUSIONS:.

1. There was no indication of a mutagenic response (a significantly increased incidence of micronucleated PCEs) at any of the SAN 619F dose levels (16.7, 55.7 and 167 mg/kg) for any of the scheduled sacrifice times (24, 48 and 72 hrs). The positive control (cyclophosphamide) elicited the appropriate response, although it is noted that the route of administration for the CP (IP injection) was not the same as for the SAN 619F (oral dosage).

2. The study is currently classified as not acceptable because the purity of the test material (identified as SAN 619 F, batch 8405) is not reported. Additionally, the supplier of the mice used in this study should be identified.

A. MATERIALS:

1. Test compound: SAN 619 F, batch 8405. Described as an off-white powder. The purity is not reported.

2. Positive control material: Cyclophosphamide (CP) at 100 mg/kg was dissolved in sterile deionized water and was used as the positive control. It was administered via intraperitoneal (IP) injection.

3. Test animals: "Adult male and female mice, strain Swiss random, from a randomly bred closed colony. It is indicated on p. 14 that these mice had been purchased, but the source is not reported.

B. STUDY DESIGN:

1. Animal assignment: Ten mice, five males and five females, made up each group. They were randomly allocated to the groups according to a standard operating procedure. The following dosages and times of sacrifice were used:

		Number of animals and times of sacrifice (hrs)					
		24 hr		48 hr		72 hr	
Group	Dose (mg/kg)	M	F	M	F	M	F
Negative control (DMSO only; oral dosage)		5	5	-	-	-	-
Positive control (CP-100 mg/kg by IP injection)		5	5	-	-	-	-
SAN 619F - oral	16.7	5	5	5	5	5	5
"	55.7	5	5	5	5	5	5
"	167.0	5	5	5	5	5	5

2. Dosage justification and administration:

From p. 6: "The oral LD50 for SAN 619 F was known to be 200 mg/kg for male mice and 218 mg/kg for female mice. Dose selection was based upon this information using 80% of the combined LD50 for both sexes 167.0 mg/kg as a top dose, one third of that, 55.7 mg/kg for the medium dose and one tenth of the top dose, 16.7 mg/kg, as the low dose."

3. Preparation of slides:

After sacrifice, tibial marrow suspensions were smeared on slides and air-dried. The slides were fixed in methanol, stained in May-Gruenwald solution followed by Giemsa, and rinsed in deionized water.

4. Slide evaluation:

From p. 15: "One thousand PCEs (polychromatic erythrocytes) per animal were scored. The frequency of micronucleated cells was expressed as percent micronucleated cells based on the total PCEs present in the scored optic field."

"The frequency of PCEs versus mature RBCs was determined by scoring the number of mature erythrocytes (RBCs) observed in the optic fields while scoring the first 1000 PCEs for micronuclei."

"For control of bias, all slides were coded prior to scoring."

5. Evaluation and Assessment:

"Micronuclei were darkly stained and generally round, although almond and ring-shaped micronuclei occasionally occur. Micronuclei had sharp borders and were generally between 1/20 and 1/5 the size of the PCE. The unit of scoring was the micronucleated cell, not the micronucleus; thus the occasional cell with more than one micronucleus was counted as one micronucleated PCE, not two (or more) micronuclei. The staining procedure permitted the differentiation by color of polychromatic and normochromatic erythrocytes (bluish-grey and red, respectively)."

"The data generated in this study was analyzed by the Student's t-test...or by other appropriate statistical tests... Individual animal results were used as data points in the analysis. The set of micronuclei frequencies among the controls was compared to the set for each treatment level. Male and female animal data were analyzed both separately and combined."

"Criteria for determining a positive response involved a statistically significant dose-related increase in micronucleated PCEs, or the detection of a reproducible and statistically significant positive response for at least one dose level. A test article that induced neither a

statistically significant dose response nor a statistically significant and reproducible increase at one dose level was considered negative. In either case the final decision was based on scientific judgment."

6. There is a Good Laboratory Practice Statement on page 3 of the report, along with a signed Q.A. Inspection statement on p. 17.

C. RESULTS:

1. Animal observations:

There is no reporting that any of the mice either showed or did not show any symptoms. However, on page 11 (table 2c) it is indicated that one high-dose (167 mg/kg) male died before the 48-hr sacrifice, and on p. 12 (table 2d) one medium dose (55.7 mg/kg) and three high-dose males died before their scheduled 72-hr sacrifice.

2. Slide evaluations:

A summarization of results is given on appended page 1 of this report. For all times of sacrifice (24, 48 and 72 hrs), but particularly for 48 and 72 hours, the high-dose (167 mg/kg) animals showed a reduced PCE/RBC ratio as compared to controls; none of these values is reported as statistically significant, but there is no indication that any statistical test was applied to these ratios.

The CP-treated animals showed a significantly increased incidence of micronucleated PCEs for both male and female groups separately, as well as for combined sexes with respect to their controls.

There was no indication of any significantly elevated incidence of micronucleated PCEs in any of groups that were orally dosed with the SAN 619F.

D. DISCUSSION:

The test material was administered at a sufficiently high enough level (as demonstrated by the mortality which occurred, particularly among high-dose males scheduled for sacrifice at 72 hours). There was no indication of a mutagenic response (a significantly increased incidence of micronucleated PCEs) at any of the SAN 619 F dose levels for any of the scheduled sacrifice times. The positive control elicited an appropriate response, although it is noted that the route of administration (IP injection) for the cyclophosphamide was not the same as that used for the SAN 619 F (oral dosage).

The study is, however, currently classified as not acceptable because the purity of the test material used is not reported. Additionally, the source of the mice should be reported (if only this information had been lacking, the study could have still been classified as acceptable).

CYPROCONAZOLE Tox review 007003

Page 187 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Byron T. Backus Byron T. Backus 12/02/88
Section 2, HFASB (TS-769C)

Secondary Reviewer: John H. S. Chen, D.V.M. John H S Chen 12/2/88
Section 1, HFASB (TS-769C)

Tertiary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/6/88
Section 2, HFASB (TS-769C)

DATA EVALUATION REPORT IV

STUDY TYPE: Unscheduled DNA Synthesis (UDS) in vitro in rat hepatocytes TOX CHEM NO. 272E

ACCESSION NUMBER: 406077-29 MRID NO:

TEST MATERIAL: SAN 619 F, 96.2%

SYNONYMS: SAN 619 F

STUDY NUMBER(S): T8028.380

SPONSOR: Sandoz AG

TESTING FACILITY: Microbiological Associates, Inc.
9900 Blackwell Road
Rockville, MD 20850

TITLE OF REPORT: Unscheduled DNA Synthesis in Rat Primary Hepatocytes Test Article SAN 619F

AUTHOR(S): Curren, R. D.

REPORT ISSUED: 04/26/88

CLASSIFICATION: Not acceptable: additional information required

CONCLUSIONS:

1. There was no indication of an increased level of incorporation of ^{3}H-TdR in rat hepatocytes as a result of exposure to SAN 619F, either as an isolated occurrence at a single dose level or as part of a dose-related trend. The dose levels that were evaluated were 0.15, 0.5, 1.5, 5 and 15 ug/ml.

2. The study has been classified as not acceptable because there is insufficient reporting as to why levels of 50, 100 and 150 ug/ml were "Too toxic to be evaluated for UDS." The only information given (p. 13) is "Microscopic examination of the hepatocyte cultures indicated toxicity at 150, 100 and 50 ug/ml and normal morphology at lower doses...the top...doses

188

of 150, 100 and 50 ug/ml were too toxic to be evaluated." Before we can accept this study we need to have additional details regarding the microscopic findings at these levels, particularly as the LDH activities indicated relative toxicities below 100% at these levels (68%, 61% and 16% respectively); also, clarification for selecting the maximum applied dose of SAN 619F for this study is required.

A. MATERIALS:

1. Test compound: SAN 619F, purity 96.2%. Supplied from SANDOZ AG. Dissolved and diluted in DMSO to make up the stock solutions. "The test article was diluted to appropriate concentrations immediately prior to use."

2. Positive control material: 7,12-dimethylbenz(a)anthracene (DMBA) from Kodak. This was tested at concentrations of 5 and 10 ug/ml.

3. Indicator cells: hepatocytes from normal adult male Fischer 344 rats.

B. STUDY DESIGN:

1. Rat hepatocytes were obtained by perfusion of the liver with a collagenase solution. "The liver was removed from the animal and the cells were dissociated, counted and seeded into 35 mm dishes containing coverslips (5×10^5 viable cells/dish). The cells were seeded in Williams' Medium E...supplemented with 10% fetal bovine serum, 2mM L-glutamine and 50 ug/ml gentamicin. The cultures were incubated at $37 \pm 1^{\circ}C$ in a humidified $5 \pm 1\%$ CO_2 incubator. After the incubation period the cultures were washed, refed with serum-free medium and used in the test."

2. Dose selection: From p. 10: "A preliminary cytotoxicity test was performed to establish an appropriate dose range ... Ten doses ranging from 0.15 to 5000 ug/ml were tested. Replicate cultures of rat hepatocytes were washed, refed with serum-free medium and treated with the test article 90-180 minutes after seeding. Eighteen to twenty hours later, an aliquot of culture fluid was removed, centrifuged, and the level of lactic acid dehydrogenase (LDH) activity in the culture fluid determined... To determine relative toxicity each treatment condition was compared to the most toxic treatment which was considered to represent 100% lysis. This was done since the normal separate measurement of 100% lysis was inadvertantly omitted. Visual observation of the treated cells confirmed this level of toxicity."

3. UDS assay: Nine dose levels (0.05, 0.15, 0.5, 1.5, 5, 15, 50, 100 and 150 ug/ml) were tested. "Ninety to 150 minutes after seeding the hepatocyte cultures were rinsed and refed with serum-free medium. Three replicate plates seeded with 5 x 10^5 rat hepatocytes/plate were then treated with 0.05 to 150 ug/ml of test article... DMSO, which was used to dissolve both the test article and DMBA, was used as the solvent control... Each test article and control dish received ^{3}H-thymidine at a final concentration of 10 uCi/ml. ...three cultures per dilution were treated with the test article and control compounds for a parallel toxicity test ...After eighteen to twenty hours of exposure, the cells in the Unscheduled DNA Synthesis assay plates were washed in serum-free WME, swelled in 1% sodium citrate and fixed in ethanol-acetic acid fixative. The coverslips were air-dried, mounted cell side up on glass slides, and allowed to dry."

"The slides were coated with Kodak NTB emulsion and stored in a refrigerator for six days in light tight boxes with desiccant. The medium control coverslips were originally mounted cell side down. They were remounted cell side up and were stored in a refrigerator for ten days in light tight boxes with desiccant. The slides were then developed in Kodak D-19 developer, fixed in Kodak fixer and stained in hematoxylin-sodium acetate-eosin stain."

"The slides were read "blind" on an Artek Colony Counter. Nuclear grains were counted in 50 cells in random areas of each of three coverslips per treatment where possible...net nuclear counts were determined by counting three nucleus-sized areas adjacent to each nucleus and subtracting the average..."

"For each treatment slide, the net nuclear counts were averaged and the standard deviation (S.D.) determined and recorded..."

4. Assay evaluation criteria:

"If the mean net nuclear count was increased by at least five counts over the control, the results for a particular dose were considered significant. A test article was judged positive if it induced a dose-related response and at least one dose produced a significant increase in the average net nuclear grains when compared to that of the control. In the absence of the dose response, a test article which showed a significant increase in the mean net nuclear grain count in at least two successive doses was

considered positive...a significant increase in the net nuclear grain count at one dose level without any dose response, the test article was considered to be equivocal. The test article was considered negative if no significant increase in the net nuclear grain counts at any dose level was observed."

5. A signed Quality Assurance Statement is provided on p. 5.

C. RESULTS:

1. Preliminary assay for cytotoxicity: The following values for viability in the preliminary cytotoxicity study are reported in table 1 (p. 14):

Dose	Corrected LDH	Relative Toxicity
DMSO 10 ul/ml (solvent control)	0.0	0%
WME (Media control)	7.0	2%
0.15 ug/ml	-35.5	-8%
0.5 ug/ml	-22.5	-5%
1.5 ug/ml	-26.5	-6%
5.0 ug/ml	-48.5	-11%
15.0 ug/ml	-60.0	-13%
50.0 ug/ml	-28.5	-6%
150.0 ug/ml	335.0	75%
500.0 ug/ml	323.5	72%
1500.0 ug/ml	348.0	78%
5000.0 ug/ml	448.0[a]	100%[a]

[a]Defined as representing 100% toxicity because the 100% LDH control was not sampled.

2. Results of UDS Assay and Parallel Cytotoxicity Assay:

The result summary of the UDS assay are given in appended page 1 (from table 3, p. 16 of the report) and of the parallel cytotoxicity assay are given in appended page 2 (from table 2, p. 15 of the report).

D. DISCUSSION:

For the evaluated dosages (range from 0.15 to 15 ug/ml) there was no indication that there was an increased level of incorporation of ^{3}H-TdR as a result of exposure to SAN 619F, either as an isolated occurrence at any of the dose levels or as part of a dose-related trend. It is noted the medium control coverslips may have been somewhat more exposed than the others, as they had (presumably) been sitting for ten days with emulsion before being developed, while the other slides were allowed to sit for only six days. However, even so, there was no indica-

tion that this protocol deviation had any significant effect on the results of the study.

In addition to the five dosage levels ranging from 0.15 to 15 ug/ml, the cells were exposed to 50, 100 and 150 ug/ml. It is reported (p. 16) that these levels were "Too toxic to be evaluated for UDS." The only information given (p. 13) is "Microscopic examination of the hepatocyte cultures indicated toxicity at 150, 100 and 50 ug/ml and normal morphology at lower doses." There is no reporting as to what the (presumably) abnormal morphology was. With the parallel cytotoxicity assay indicating (from LDH activities) only 16% relative toxicity at 50 ug/ml, 61% at 100 ug/ml, and 68% at 150 ug/ml, we need to have additional details regarding the microscopic (cellular morphology?) findings before we can accept the statement that these concentrations were "Too toxic to be evaluated for UDS."

Based on the results of the cytoxicity assays for the test substance presented in Tables 1 and 2, relative toxicity (RT-LDH release) of 100% was observed at the test concentrations higher than 5000 ug/ml, but it is noted that considerable variations of RT values were observed at the concentrations of 50 ug/ml and 15 ug/ml (i.e., -6% at 50 ug/ml, and -13% at 15 ug/ml in Table 1; 16% at 50 ug/ml, and 3% at 15 ug/ml in Table 2). It is questionable whether an appropriate upper limit of SAN 619 F based on these results was selected for this study. Clarification for selecting the maximum applied dose of the test compound on the basis of these cytotoxicity findings is also required.

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 193 through 194 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007003

Reviewed by: Byron T. Backus Byron T. Backus 12/07/88
Section 2, HFASB (TS-769C)

Secondary Reviewer: John Chen, D.V.M. Richard H. Chen 12/8/88
Section 1, HFASB (TS-769C)

Tertiary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/14/88
Section 2, HFASB (TS-769C)

DATA EVALUATION REPORT VI

STUDY TYPE: Mutagenicity - In vitro cell transformation with Syrian Hamster Embryo (SHE) cells

TOX CHEM NO. 272E

ACCESSION NUMBER: 406077-24

MRID NO:

TEST MATERIAL: SAN 619F

SYNONYMS:

STUDY NUMBER(S): Laboratory Project LMP 099C

SPONSOR: Sandoz

TESTING FACILITY: Laboratorium fur Mutagenitasprunfung
Technische Hochschule
Darmstadt, Germany

TITLE OF REPORT: SAN 619F: In vitro cell transformation with Syrian Hamster Embryo (SHE) cells

AUTHOR(S): Miltenburger, H. G.

REPORT ISSUED: 02/26/85
02/24/85 (study completion date)

CLASSIFICATION: Acceptable

CONCLUSIONS:.

1. There was no transformation of the SHE cells from exposure to SAN 619F for 6 or 48 hours without metabolic (S9) activation, or as a result of 6-hr exposure to SAN 619F in the presence of rat S9 mix. Dose levels used were 20, 50, 100 and 200 ug/ml; although there was no evidence for cytotoxicity at any of these dose levels in a preliminary assay, the test material precipitated out at concentrations greater than 200 ug/ml. Transformation was elicited by the positive controls, although we would have anticipated that the 48-hr exposure to MNNG would have given more transformed cells per 1000 colonies than

the 6-hr exposure. There is also some uncertainty regarding the exposure period in the preliminary cytotoxicity study (it is assumed that cells were exposed for 6 hours in the presence of S9, but it is uncertain whether they were exposed for 6 or 48 hours without S9).

2. However, even if clarifications are made regarding the exposure period to SAN 619F in the preliminary cytotoxicity study, this would make no difference in the essential finding of the study, which was that SAN 619F was negative for mutagenic activity in this assay system. The study is therefore classified as acceptable.

A. MATERIALS:

1. Test compound: SAN 619 F, no batch no. reported. Purity reported as 94.4%. No physical description given in this report.

2. Positive control materials: N-methyl-N'-nitro-N-nitroso-guanidine (MNNG), at 0.5 ug/ml of medium, used without metabolic (S9) activation. Benzo(a)pyrene (BaP) at 25 ug/ml of medium, used with metabolic (S9) activation.

3. Test cells: Cell suspensions were prepared by mincing and trypsinizing 12-14 day old Syrian hamster embryos. These cells were inoculated into 75 cm^2 plastic flasks at a density of 4-5 x 10^6 cells/flask. After several days incubation the cell monolayers became confluent. "The cells are then trypsinized washed once with phosphate buffered saline and suspended in MEM with 7.5% DMSO. 2 ml samples of 2.5 x 10^6 cells/ml or 5 x 10^6 cells/ml are filled into cryotubes...and frozen down to -196° C as stock cultures. The stock cultures with 2.5 x 10^6 cells/ml are used for target cells and those with 5 x 10^6 cells/ml for feeder cells."

4. S9/S9 mix: Prepared from the livers of five 8-12 weeks old male Wistar rats which had been previously given a single IP injection of 500 mg/kg Aroclor. The resulting preparation was stored at -20° C. The S9 mix was prepared by thawing the preparation and mixing with cofactor. The following were the final concentrations in the S9 mix: 0.3 mg/ml protein (from S9); 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate; 5 mM NADP and 100 mM sodium-ortho-phosphate buffer, pH 7.4.

B. STUDY DESIGN:

1. Preliminary cytotoxicity:

From p. 16: "The toxicity of the test substance was determined in a pre-experiment... According to the results from this pre-experiment the four concentrations to be applied in the transformation assay were chosen."

The period of the exposure to the test substance in this preliminary cytotoxicity study is not reported.

2. Dosages used:

The following dosages and exposure conditions were used:

Group	6-hr	48-hr	6-hr + S9 mix
Negative control (untreated)	x	x	x
Negative (cells with solvent)	x	x	x
Positive control			
MNNG	x	x	-
BaP	-	-	x
20 ug/ml SAN 619F	x	x	x
50 ug/ml SAN 619F	x	x	x
100 ug/ml SAN 619F	x	x	x
200 ug/ml SAN 619F	x	x	x

3. Experimental design:

From p. 17: "Feeder cells were seeded... After two or three days they were trypsinized (0.2% trypsin in Ca-Mg-free balanced salt solution). The action of trypsin was stopped by dilution with nutrient medium (1:5). After centrifugation the cell pellet was resuspended in phosphate buffered saline (PBS) and irradiated with 5000 rad x-rays...at room temperature. After irradiation the cell density per ml was determined by a particle counter... Each 1.5 x 10^5 cells were seeded into five 80 cm^2 plastic flasks...with 7 ml of MEM + 20% FCS. 5000 target cells were seeded 24 h later into the same flasks with additional 7 mm of medium (MEM + 20% FCS). After another 24 h the four...concentrations of the test substance were added to the flasks. The final amount of medium was then 28 ml/flask. For metabolic activation S9 mix was added (0.3 mg protein/ml medium) into parallel flasks. A medium change was made in the flasks with S9 mix (controls and treated cultures) 6 h after the beginning of the treatment; in the flasks without S9 mix 48 h after the beginning of the treatment. The new medium was free of test substance and S9 mix. All incubations were done at 37^{o} C in an humidified atmosphere with 5% CO^2. Eleven days after seeding of the target cells the colonies were counted and scored for cell transformation."

"The colonies from the target cells can clearly be identified because no colonies are formed by the feeder cells. After fixation with Carnoy's fixative the colonies were stained with GIEMSA solution...and analysed by light microscopy."

4. Evaluation:

From p. 20: "The analysis of colonies for the occurrence of morphologically altered cells was done by using ZEISS and LEITZ microscopes. 1000 colonies were scored per test substance concentration and in the controls... A colony was defined as being transformed when the cells piled up three dimensionally...and when they showed criss/cross growth in the marginal zone of the colony."

"A chemical is considered negative when it does not induce a transformed colony in each sample of 200 colonies from 5 parallel flasks, i.e. in 1,000 colonies per concentration, both without and with metabolic activation."

From p. 36: "In all reports on the SHE cell transformation system so far there is no evidence for spontaneous tranformation of SHE cells under the experimental conditions as described in this protocol. However, in such a case it could be advisable to repeat the experiment with the concentration in question and to enlarge the sample size to find out whether it is a reproducible effect produced by the test substance. This procedure should be considered ...especially when the transformed colony is found in the group treated with the highest concentration of the test substance."

5. There is a signed (but undated) statement that "this study was performed in compliance with Good Laboratory Practice standard which meet the requirements for 40 CFR Part 160." on page 3 of the report.

C. RESULTS:

1. Preliminary cytotoxicity:

From p. 16 (it is noted that the period of exposure to the test substance is not reported):

Concentration of test substance ug/ml	% relative survival without S9 mix	with S9 mix
0 = solvent	11.0*=100	10.3*=100

0.01	98	107
0.1	98	100
1.0	104	99
10.0	97	100
100.0	90	87
200.0	90	84

* = absolute survival
The test substance precipitated at concentrations higher than 200 ug/ml.

2. Numbers of transformed colonies:

From p. 21:

Group	Transformed colonies (%) 6-hr	48-hr	6-hr + S9 mix
Negative control (untreated)	0.0	0.0	0.0
Negative (cells with solvent)	0.0	0.0	0.0
Positive controls			
MNNG (0.5 ug/ml)	1.1	0.9	-
BaP (25 ug/ml)	-	-	1.3
Test substance			
20 ug/ml SAN 619F	0.0	0.0	0.0
50 ug/ml SAN 619F	0.0	0.0	0.0
100 ug/ml SAN 619F	0.0	0.0	0.0
200 ug/ml SAN 619F	0.0	0.0	0.0

D. DISCUSSION:

There was no transformation of the SHE cells as a result of exposure to SAN 619F for 6 or 48 hours without metabolic (S9) activation, or as a result of 6-hr exposure to SAN 619F in the presence of rat S9 mix. The dose levels used were 20, 50, 100 and 200 ug/ml; although there was no evidence for cytotoxicity at any of these dose levels in a preliminary assay, the test material precipitated out at concentrations greater than 200 ug/ml. Transformation was elicited by the positive controls, although it is noted that we would have anticipated that the 48-hr exposure to MNNG would have given more transformed cells per 1000 colonies than the 6-hr exposure. There is also some uncertainty regarding the exposure period in the preliminary cytotoxicity study (it is assumed that cells were exposed for 6 hours in the presence of S9, but it is uncertain whether they were exposed for 6 or 48 hours without S9).

However, it is concluded that even if clarifications are made regarding the exposure period to SAN 619F in the preliminary cytotoxicity study, this would make no difference in the essential finding of the study, which was that SAN 619F was negative for mutagenic activity in this assay system. The study is therefore classified as acceptable.

Reviewed by: Byron T. Backus Byron T. Backus 12/12/88
Section 2, HFASB (TS-769C)

Secondary Reviewer: John Chen, D.V.M. John Chen 12/13/88
Section 1, HFASB (TS-769C)

Tertiary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/14/88
Section 2, HFASB (TS-769C)

DATA EVALUATION REPORT VII

STUDY TYPE: Mutagenicity - Mitotic non-disjunction assay with Saccharomyces cerevisiae strain D6 TOX CHEM NO. 272E

ACCESSION NUMBER: 406243-07 MRID NO:

TEST MATERIAL: SAN 619F

SYNONYMS:

STUDY NUMBER(S): E-9334; report no. 6313/85

SPONSOR: Sandoz

TESTING FACILITY: Litton Bionetics
Landjuweel 11
3905 Pe Veenendaal
The Netherlands

TITLE OF REPORT: Mutagenicity Evaluation of SAN 619F in the Mitotic Non-Disjunction Assay with Saccharomyces Cerevisiae Strain D_6

AUTHOR(S): Hoorn, A. J. W.

REPORT ISSUED: 01/11/85 (study completion date)

CLASSIFICATION: Without S9 activation: not acceptable without additional information
With S9 activation: not acceptable

CONCLUSIONS:.

1. There was no indication of an increased absolute number of cycloheximide-resistant colonies or of an increased incidence of aneuploids among these resistant colonies following "overnight" exposure to SAN 619F at concentrations of 10, 100, 250, 400, 500 or 550 ug/ml. The positive control EMS, tested only in the absence of S9, elicited a positive response, as demonstrated both by an increase in absolute numbers of cycloheximide-resistant colonies, and an increased incidence of aneu-

ploids among these colonies. The concentration range for the test compound included doses at which there was no, moderate, and nearly complete cytotoxicity, both in the presence and absence of S9.

2. The major difficulty in accepting the complete findings of this study is due to the lack of a positive control in the presence of S9. The report states (p. 10) that "In the activation part no positive control compound was included because there is no acceptable positive control available for this part of the assay." However, this has resulted in uncertainty as to whether or not the S9 mixture in this assay with S. cerevisiae D6 was sufficiently active to produce a mutagenic response under the appropriate circumstances.

3. The findings of the part of the study conducted without S9 will be acceptable provided information is submitted as to the period (in hours) that the cells were exposed to the test substance. It is noted that the protocol (p. 24) states the exposure would be for 3 hours at 37° C, but the report (p. 9) states that the mixture containing the test material "was incubated overnight at 30° C." Additionally, we should have information regarding the purity of the test material.

A. MATERIALS:

1. Test compound: SAN 619F, batch 8405. Purity: not reported. Physical description: a white powder (received Nov. 13, 1984). Dimethylsulfoxide (DMSO) was used as the solvent for the SAN 619F and for the serial dilutions.

2. Positive control material: "Ethylmethanesulfonate (EMS) was used as the positive control for the nonactivation part. In the activation part no positive control compound was included because there is no acceptable positive control available for this part of the assay.

201

3. Indicator Organism: Saccharomyces cerevisiae strain D_6, a diploid yeast strain with the following genotype:

Chromosome III	his 4			a		
	+			alpha		
Chromosome VII	ade 3		leu 1	trp 5	cyh 2	met 13
	+		+	+	+	+
Chromosome XV	ade 2-40					
	ade 2-40					

"The strain D_6...carries a series of recessive and coupled markers on chromosome VII and when plated on the appropriate growth medium produces red colonies which are sensitive to the presence of 2 ug/ml cycloheximide in the growth medium. Loss of chromosome VII carrying the wildtype alleles, presumably by non-disjunction, results in the production of white (ade 3) colonies resistant to cycloheximide. The resistant, white colonies are then tested by replica plating on minimal media lacking the amino acids leucine, tryptophan or methionine to verify that the complete chromosome VII, carrying the wild type alleles, was lost."

4. S9/S9 mix: "A 9,000 x g supernatant prepared from Sprague-Dawley adult male liver induced by Aroclor 1254...was purchased from Litton Bionetics, Biological Products, and used in this assay." The S9 mix had the following composition per ml: NADP (sodium salt), 4 umoles; D-glucose-6-phosphate 5 umoles; $MgCl_2$, 8 umoles; KCl, 33 umoles; sodium phosphate buffer (pH 7.4) 100 umoles; organ homogenate from rat liver (S9 fraction) 100 uliters.

B. STUDY DESIGN:

1. Preliminary toxicity:

2 ml of yeast cells (about 1.5×10^8 cells/ml), along with 0.9 - 0.95 ml pH 7.4 phosphate buffer and 0.05 - 0.1 ml of a solution of the test compound to give the appropriate dose were added to a sterile vial. "The mixture was incubated overnight at 30° C in a rotary shaker. After incubation, survival was determined by adding aliquots of an appropriate dilution of the suspension...to 2 ml of molten

(45° C) overlay agar which was poured onto the respective yeast complete plates. For each experimental point 3 plates were used. These plates were incubated for approximately 2 days and scored."

2. Non-disjunction assay:

From p. 8: "Doses used in the actual assay were selected from a preliminary toxicity test performed on strain D_6." These were 0 (solvent control), 10, 100, 250, 400, 500 and 550 ug/ml SAN 619F both with and without S9. In addition, 0.1% and 0.25% EMS were tested (in the absence of S9) as positive controls.

In the nonactivation assay, 2 ml of yeast cells (about 1.5×10^8 cells/ml), along with 0.95 ml phosphate buffer (pH 7.4) and 0.05 ml of a solution of the test compound were added to a sterile vial. This mixture was incubated overnight at 30° C in a rotary shaker. Suspensions were then diluted with 7 ml pH 7.4 phosphate buffer and centrifuged (2000 rpm) for 10 minutes. The supernatant was discarded and the pellet was resuspended in 3 ml phosphate buffer (pH 7.4). From p. 9: "These suspensions were used to assay for cycloheximide resistance and cell survival as follows:"

"Aliquots of the suspension were placed in 2 ml molten overlay (at 45°C) and poured on medium with cycloheximide."

"Aliquots of an appropriate dilution of the suspension were placed in 2 ml of overlay agar and poured onto yeast complete plates."

"Aliquots and dilutions were based on haemacytometer counts of the respective solvent controls in order to transfer approximately 2×10^5 cells to each of ten selective plates and 200 cells to each of three complete plates for each experimental point."

"The complete and selective plates were incubated for approximately 2 days and 10-14 days respectively. Then they were counted."

"All white colonies growing on the selective plates (presumptive aneuploids) were resuspended in 0.5 ml of a 0.2M phosphate buffer, pH 7.4. Ten microliters of these suspensions were then replica plated on selective plates for leucine, tryptophan and/or methionine requirement, respectively. These plates were incubated for 6-9 days and then analyzed."

"An additional sample was transferred to complete plates to confirm that yeast cells had been plated onto the selective plates. These plates were incubated for 2-3 days and then analyzed."

"The activation assay was run concurrently with the nonactivation assay. The only difference was the addition of 0.95 ml of S9 mix to the vials in place of the phosphate buffer which was added in the nonactivation assay."

4. Evaluation:

From p. 10: "The test measures surviving populations and the induction of mitotic nondisjunction concurrently. Therefore, it is quantitative in nature and the data are expressed as a frequency (mutants per survivor)."

"The demonstration of dose-related increases is an important criterion in establishing mutagenicity..."

"The yeast strain D_6 is a diploid strain of Saccharomyces cerevisiae...used for studying chromosomal non-disjunction during mitosis. This chromosomal non-disjunction leads to aneuploidy which in turn causes phenotypic as well as genetic effects. If the solvent control value is within the normal range, a test article that produces a positive dose-response over three concentrations with the highest increase equal to twice the solvent control will be considered positive."

"If a test article produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data lose significance."

5. There is a signed and dated combined Good Laboratory Practice and Q.A. Inspection Statement on p. 3 which appears to be the same document (with added signatures) as the Q.A. Statement on p. 20.

C. RESULTS:

1. Preliminary cytotoxicity:

The following is from p. 14:

TEST COMPOUND ug PER ML	SURVIVING POPULATIONS PER 0.50 ml**	PERCENT SURVIVAL RELATIVE TO CONTROL
0 (control)*	405	100.0
1.22	560	138.3
2.44	425	104.9
4.88	442	109.1
9.77	391	96.5
19.53	395	97.5
39.06	395	97.5
78.13	373	92.1
156.25	359	88.6
312.5	319	78.8
625	0	0.0

At 625 ug/ml and higher (1250, 2500, 5000 and 10000 ug/ml) there was complete cytotoxicity.

* Solvent control: 100 ul per 3 ml
** Total of 3 plates at 10^{-5} dilution.

2. Induction and analysis of presumptive aneuploid cells:

There was no evident dose-related increase in absolute numbers of cycloheximide resistant colonies relative to the controls as a result of exposure to SAN 619F either in the presence or absence of S9. The positive controls showed a 3-4X increase (positive controls were tested only in the absence of S9). Refer to appended page 1 (from table 3). Analysis of the colonies (refer to appended page 2) confirmed that a total of 6 (solvent control: 1/23; 0.1% EMS: 2/88, and 0.25% EMS: 3/110) were aneuploid, with no growth on media lacking methionine, tryptophan and leucine. There were no such colonies among those which had been exposed to the SAN 619F.

D. DISCUSSION:

There was no indication of an increased absolute number of cycloheximide-resistant colonies or of an increased incidence of confirmed aneuploids among these resistant colonies following exposure to SAN 619F at concentrations of 10, 100, 250, 400, 500 or 550 ug/ml. The positive control EMS, tested only in the absence of S9, elicited a positive response, as demonstrated both by an increase in

absolute number of cycloheximide-resistant colonies, and an increased incidence of confirmed aneuploids among these colonies. The concentration range for the test compound included doses at which there was no, moderate, or nearly complete cytotoxicity, both in the presence and absence of S9.

The major difficulty in accepting the complete findings of this study is from the lack of a positive control in the presence of S9. The report states (p. 10) that "In the activation part no positive control compound was included because there is no acceptable positive control available for this part of the assay." However, this has resulted in uncertainty as to whether or not the S9 mixture in this assay with S. cerevisiae D6 was sufficiently active to produce a mutagenic response under the appropriate circumstances. This part of the assay has been classified as not acceptable.

It is concluded that the part of the study conducted in the absence of S9 activation will be acceptable, provided information is submitted as to the period (in hours) that the cells were exposed to the SAN 619F. It is noted that the protocol (p. 24) states the exposure would be for 3 hours at 37° C, but the report (p. 9) states that the mixture containing the test material "was incubated overnight at 30° C." Additionally, we should have information regarding the purity of the test material.

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 207 through 208 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel K. Clark Swentzel 12/5/88 007003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 12/9/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity TOX. CHEM. NO.: 272E

MRID NO.: 406069-05

TEST MATERIAL: SAN 619F 40 WDG [40% alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol]
SYNONYMS: A.I.[Cyproconazole; SAN 619F]

STUDY NUMBER(S): 8658D/SNC 22/AC

SPONSOR: Sandoz Corp.

TESTING FACILITY: Huntingdon Research Centre, Ltd.

TITLE OF REPORT: Acute Oral Toxicity to Rats of SAN 619F 40 WDG

AUTHOR(S): J.R. Gardner

REPORT ISSUED: January 6, 1988

TEST DATES: December 16, 1987 - January 31, 1988

CONCLUSIONS

The acute oral LD_{50} and the respective 95% confidence limits for SAN 40 WDG were estimated to be:

Males and females combined:	1,010 (830 to 1,240) mg/kg.
Males only:	780 (620 to 980) mg/kg.
Females only:	1,340 (1,070 to 1,690) mg/kg.

Tox. category = III

Core-classification: minimum

Quality assurance statement: signed and dated by the QAU

209

007003

Test Material

SAN 619F 40 WDG (40% a.i.), batch no. 6059, tan powder, vehicle: distilled water(various concentrations prepared to maintain a constant dosage volume of 10 ml/kg)

Test Animals

Male and female Charles River [Crl:CD(SD)BR] rats, 99-144g, 4-6 weeks of age, 5/sex/group, acclimated 7 days prior to the study

Feed and Water

A standard laboratory diet (Labsure LAD 1) and water were provided ad libitum. Access to food was prevented overnight prior to and approximately 4 hours after dosing.

Housing

The rats were housed in groups (5 each) by sex in metal cages with wire mesh floors.

Identification

Individually by cage number and ear punching.

Environmental Parameters

Temperature range: 21-23°C, mean relative humidity: 50%, room air exchange rate: 13 changes/hour, light cycle: 12/12

PROCEDURES:

Limit test

One group of 5 male and 5 female rats was given a single oral dose of the test substance at 5.0 g/kg body weight.

Results

All treated rats died.

Range-finding test

Four groups of 2 male and 2 female rats were treated at oral dose levels of 0.10, 0.50, 1.00 and 2.50 g/kg body weight and observed for 5 days.

Results

The results of the limit test and range-finding studies indicated that the acute median lethal oral dose of test material was between 0.5 and 1.00 g/kg for both male and female rats (mortality data: Appended page 1).

Main test

Dosing

Based on the results of the range-finding study, a single dose of test material was administered via oral gavage to groups of 5 rats/sex at dosages of 0.50, 0.80, 1.26 and 2.00 g/kg with a constant dosage volume of 10 ml/kg.

210

217

Observations

The animals were observed frequently during the day of dosing and twice daily for the remainder of the 14-day observation period. Information recorded during this study included: the approximate time of death of individual rats; the nature, severity, approximate time of onset and duration of each toxic sign; individual body weights on days 1 (day of dosing), 8 and 15 and at death.

Post mortem examination

Rats that were alive at the end of the observation period were sacrificed on day 15. All animals in the study were necropsied and subjected to gross examinations.

STATISTICAL ANALYSIS

The LD_{50} was calculated for male and female rats by the method of Weil (Biometrics 8, 249, 1952).

Results

Mortality (data on Appended page 1)

Deaths occured among male rats at 0.80 g/kg and above and among female rats at 1.26 g/kg and above. All rats administered 2.0 mg/kg died on days 2 and 3. One male in the 1.26 g/kg group was sacrificed moribund on day 4.

Clinical signs (data on Appended page 2)

The investigator indicated that signs of reaction to treatment, observed within 10 minutes of dosing were pilo-erection in all animals and abnormal body carriage (hunched posture), abnormal gait and pallor of the extremities in rats dosed a 2.0 g/kg. These signs as well as decreased respiration and lethargy were observed in all treated rats later on day 1. Other noted clinical signs were: ptosis among males dosed at 0.5 and 0.8 g/kg and all rats treated at higher doses; ataxia in all rats except 1 low-dose male and prostration from day 2 among rats that were found dead on days 2, 3, or 4. Indications of recovery were apparent on days 4 (0.5 g/kg), 4 or 5 (0.8 g/kg), and 6 (1.26 g/kg).

Body weight (data on Appended page 3)

Body weight gain was slightly inhibited during the first week of the study for 2 females at 1.26 g/kg, 2 males at 0.8 g/kg and 3 males at 0.5 g/kg; 1 low-dose female gained less body weight between days 8 and 15.

Necropsy

The investigator indicated that no gross abnormalities were found.

CONCLUSION

The acute oral LD_{50} and the respective 95% confidence limits for SAN 40 WDG were estimated to be:

Males and females combined:	1,010 (830 to 1,240) mg/kg.	Tox. category = III
Males only:	780 (620 to 980) mg/kg.	
Females only:	1,340 (1,070 to 1,690) mg/kg.	

Core-classification: minimum

211

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 212 through 214 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel K. Clark Swentzel 12/5/88 007003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 12/8/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity TOX. CHEM. NO.: 272E

MRID NO.: 406069-06

TEST MATERIAL: SAN 619F 40 WDG [40% alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol]
SYNONYMS: A.I.[Cyproconazole; SAN 619F]

STUDY NUMBER(S): 871821D/SNC 23/AC

SPONSOR: Sandoz Corp.

TESTING FACILITY: Huntingdon Research Centre, Ltd.

TITLE OF REPORT: Acute Dermal Toxicity to Rats of SAN 619F 40 WDG

AUTHOR(S): J.R. Gardner

REPORT ISSUED: December 10, 1987

TEST DATES: November 26 - December 10, 1987

Conclusion

Under the conditions of this study, the acute lethal dermal dose of SAN 619F 40 WDG to rats was found to be greater than 2,000 mg/kg, the only dosage level administered.

Tox. category = III

Core-classification: minimum

Quality assurance statement: signed and dated by the QAU

Test Material

SAN 619F 40 WDG (40% a.i.), batch no. 6059, tan powder, vehicle: distilled water(100% w/v paste, prepared on the day of dosing).

Test Animals

Male and female Charles River [Crl:CD(SD)BR] rats, 239-300g, 7-10 weeks of age, 5/sex, acclimated 15 days prior to the study

Feed and Water

A standard laboratory diet (Labsure LAD 1) and water were provided ad libitum.

Housing

The rats were housed individually in metal cages with wire mesh floors.

Identification

Individually by cage number and ear punching.

Environmental Parameters

Temperature range: 21-22°C, mean relative humidity: 58%, room air exchange rate: 15 changes/hour, light cycle: 12/12

PROCEDURES

Treatment

Ten rats (5/sex) were administered SAN 619F 40 WDG at 2.0 g/kg body weight. One day prior to treatment , hair was clipped from the dorso-lumbar region of each rat exposing an area equivalent to 10% of the total body surface. The test material was applied by spreading it over the prepared skin. The treated area (approximately 50 x 50 mm) was then covered with gauze which was held in place with an impermeable dressing encircled around the trunk. The dressings were removed at the end of the 24-hr exposure period and the treated area of skin was decontaminated with warm (30-40°C) water and blotted dry with absorbent paper.

Observations

The animals were observed frequently during the day of dosing and twice daily for the remainder of the 14-day observation period. Information recorded during this study included: the approximate time of death of individual rats; the nature, severity, approximate time of onset and duration of each toxic sign; individual body weights on days 1 (day of dosing), 8 and 15 and at death.

The treated areas of skin were examined daily for signs of dermal irritation and assessed according to the scoring system shown on Appended page 1.

Post mortem examination

Rats that were alive at the end of the observation period were sacrificed on day 15. All animals in the study were necropsied and subjected to gross examinations.

Results

Mortalities

None of the rats in this study died during the observation period.

Clinical signs

No clinical signs of systemic toxicity were observed.

Dermal reactions (data on Appended page 2)

No irritation reactions or other dermal changes were noted.

Body weights (data on Appended page 3)

All test animals gained body weight during the observation period; no concurrent controls were included for comparison.

Necropsy

No gross abnormalities were noted

Conclusion

Under the conditions of this study, the acute lethal dermal dose of SAN 619F 40 WDG to rats was found to be greater than 2,000 mg/kg, the only dosage level administered.

Tox. category = III

Core-classification: minimum

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 218 through 219 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel K. Clark Swentzel 12/9/88
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 12/9/88
Section 2 , Tox. Branch (TS-769C)

087083

DATA EVALUATION REPORT

STUDY TYPE: Acute Ocular Irritation TOX. CHEM. NO.: 272E

MRID NO.: 406069-07

TEST MATERIAL: SAN 619F 40 WDG [40% alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol]
SYNONYMS: A.I.[Cyproconazole; SAN 619F]

STUDY NUMBER(S): 8816D/SNC 25/SE

SPONSOR: Sandoz Corp.

TESTING FACILITY: Huntingdon Research Centre, Ltd.

TITLE OF REPORT: Irritant Effects on the Rabbit Eye of SAN 619F 40 WDG

AUTHOR(S): Michael Liggett

REPORT ISSUED: December 21, 1987

TEST DATES: December 7 - December 21, 1987

Conclusion

Ocular administration of 0.1 ml (57 mg) SAN 619F 40 WDG did not induce a positive irritation reaction in any of 6 treated rabbits.

Core-classification: minimum

Tox. category = IV

Quality assurance statement: signed and dated by the QAU

Test Material

SAN 619F 40 WDG (40% a.i.), batch no. 6059, tan powder.

Test Animals

Six male New Zealand white rabbits, 2.7-3.2 kg, 12-14 weeks of age, rabbits were all acclimated to the laboratory (acclimation period not given).

Feed and Water

SDS Standard Rabbit Diet and tap water were provided ad libitum.

Housing

The rabbits were individually housed in metal cages with perforated floors.

Identification

Individually by ear tag.

Environmental Parameters

Temperature: 19°C, relative humidity: 30-70%, room air exchange rate: 19 changes/hour, light cycle: 12/12

PROCEDURES

Treatment

The eyes of each animal were examined prior to instillation of the test material to determine if there was pre-existing corneal damage or conjunctival inflammation (method of examination not given).

A 57 mg amount of SAN 619F 40 WDG, the weight occupying a volume of 0.1 ml, was placed into the lower everted lid of 1 eye of each animal; the eyelids were then held together for 1 second before releasing. One untreated eye of each animal served as a control.

Observations and scoring

The eyes were examined after 1 hour and 1, 2, 3, 4 and 7 days after dosing. Grading and scoring of the treated eyes were performed using the numerical scoring system shown on Appended pages 1 and 2.

All rabbits were also observed daily for signs of ill health or toxic signs.

Results

The investigator indicated there were no signs of ill health or systemic toxicity in any of the animals.

Ocular reactions (F.I.F.R.A. guideline scores on Appended page 3; Draize system scores on Appended pages 4 and 5)

None of the animals gave a positive ocular irritation response.

221

No corneal damage or iridial inflammation was observed.

Slight redness (score = 1) of the conjunctiva was observed in all six treated eyes; in 4 of the animals at the 1 hour reading only. Slight swelling of the conjunctiva (score = 1) was observed in 3 animals; in 2 of these animals at the 1 hour reading only. A score of 1 is not considered a positive reaction for either of these changes. Slight conjunctival discharge (score = 1) was observed in all treated eyes; in 5 animals at 1 hour only.

All eyes were apparently normal 2 or 3 days after instillation of the test material.

Conclusion

Ocular administration of 0.1 ml (57 mg) SAN 619F 40 WDG did not induce a positive irritation reaction in any of 6 treated rabbits.

Tox. category = IV

Core-classification: minimum

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 223 through 227 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: K. Clark Swentzel 12/9/88 0002003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 12/9/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Irritation TOX. CHEM. NO.: 272E

MRID NO.: 406069-08

TEST MATERIAL: SAN 619F 40 WDG [40% alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol]
SYNONYMS: A.I.[Cyproconazole; SAN 619F]

STUDY NUMBER(S): 871718D/SNC 24/SE

SPONSOR: Sandoz Corp.

TESTING FACILITY: Huntingdon Research Centre, Ltd.

TITLE OF REPORT: Irritant Effects on Rabbit Skin of SAN 619F 40 WDG

AUTHOR(S): Michael Liggett

REPORT ISSUED: December 4, 1987

TEST DATES: December 1 - December 4, 1987

CONCLUSION

A single semi-occlusive application of 0.5 g of SAN 619F 40 WDG to intact rabbit skin for 4 hours induced slight dermal irritation which was not evident at 72 hours post application. This reaction does not indicate that the product is a potential dermal irritant.

Tox. category = IV

Core-classification: minimum

Quality assurance statement: signed and dated by the QAU

Test Material

SAN 619F 40 WDG (40% a.i.), batch no. 6059, tan powder.

Test Animals

Six female New Zealand white rabbits, 2.6-3.3 kg, 11-15 weeks of age, rabbits were all acclimated to the laboratory (acclimation period not given).

Feed and Water

SDS Standard Rabbit Diet and tap water were provided ad libitum.

Housing

The rabbits were individually housed in metal cages with perforated floors.

Identification

Individually by ear tag

Environmental Parameters

Temperature: 19°C, relative humidity: 30-70%, room air exchange rate: 19 changes/hour, light cycle: 12/12

PROCEDURES

Treatment

The hair was clipped from the dorso-lumbar region of each rabbit approximately 24 hours prior to application of the test material, exposing a skin area of approximately 10 cm^2.

A 0.5 g dose of test material was applied under a 2.5 cm^2 pad moistened with 0.5 ml distilled water to one intact skin site on each animal. Each treatment site was occluded with an elastic adhesive dressing for a 4 hour period. The animals remained in their cages unrestrained during the exposure period. At the end of the exposure period, the semi-occlusive dressing and gauze pad were removed and the treatment site was washed using water to remove any residual test substance.

Observations and scoring

All rabbits were observed daily for signs of ill health or toxic signs.

The treated skin was examined on day 1 (approximately 30 minutes after removal of the patches) and on days 2, 3 and 4. These sites were graded and scored using the scoring system shown on Appended page 1.

RESULTS

The investigator indicated that there were no signs of ill-health or systemic toxicity in any of the animals.

229

Dermal reactions (Appended page 2)

Very slight erythema with or without very slight edema (score = 1 for each) was observed for 3 of the animals on day 1. The remaining 3 rabbits showed very slight edema only. Very slight erythema was observed in 4 animals on day 2. All treated skin sites appeared to be normal at 72 hours.

CONCLUSION

A single semi-occlusive application of 0.5 g of SAN 619F 40 WDG to intact rabbit skin for 4 hours induced slight dermal irritation which was not evident at 72 hours post application. This reaction does not indicate that the product is a potential dermal irritant.

Tox. category = IV

Core-classification: minimum

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 231 through 232 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

006783

Reviewed by: K. Clark Swentzel K. Clark Swentzel 12/7/88
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 12/8/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Delayed Contact Hypersensitivity TOX. CHEM. NO.: 272E

MRID NO.: 406069-09 (test material); 406069-10 (positive control)

TEST MATERIAL: SAN 619F 40 WDG [40% alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol]

SYNONYMS: A.I.[Cyproconazole; SAN 619F]

STUDY NUMBER(S): 882598D/SNC 26/SS

SPONSOR: Sandoz Corp.

TESTING FACILITY: Huntingdon Research Centre, Ltd.

TITLE OF REPORT: Delayed Contact Hypersensitivity in the Guinea-pig with SAN 619F 40 WDG

AUTHOR(S): Sheena Kynoch and Brenda Parcell

REPORT ISSUED: February 29, 1988

TEST DATES: January 20 - February 29, 1988

CONCLUSION

Repeated topical applications of SAN 619F 40 WDG (50% w/w in distilled water) on guinea pigs did not induce delayed contact hypersensitivity under the conditions of this study.

Core-classification: minimum

Quality assurance statement: signed and dated by the QAU

233

Test Material

SAN 619F 40 WDG (40% a.i.), batch no. 6059, tan powder; vehicle: distilled water; dosing solution: 50% (w/w) test material in noted vehicle.

Positive control

Formalin: 25% (v/v) in distilled water for induction; 15% (v/v) in distilled water for challenge.

Test Animals

Forty female Hartley/Dunkin albino guinea pigs, 326-395 g body weight, which were acclimated to the laboratory (acclimation period not given), were used in this study. Ten animals were randomly allocated to each of the following groups:

1/ Test- SAN 619F 40 WDG
2/ Control- induction with distilled water, challenge with test material
3/ Positive control- Formalin
4/ Formalin control- induction with distilled water, challenge with formalin

Feed and Water

Vitamin C-enriched Guinea-Pig Diet F.D. 1 and tap water were provided ad libitum.

Housing

The guinea pigs were housed in suspended cages (no./cage not given) with wire mesh floors.

Identification

Individually by ear tattoo.

Environmental Parameters

Temperature: 21°C, relative humidity: 30-70%, room air exchange rate: 15 changes/hour, light cycle: 12/12.

PROCEDURES

Preliminary investigations

Test material

The investigator examined a range of dilutions of test material to determine concentrations which would cause minimal irritation, for the induction phase, and no irritation, for the challenge phase.

The investigator indicated that 50% (w/w in distilled water) was chosen because it was the maximum practical concentration that could be prepared and dosed. This concentration was used for the induction as well as the first and second challenge doses.

234

Positive control
The investigator chose 25% formalin (v/v in distilled water) for the induction doses and a concentration of 15% for the first and second challenge doses based on historical control data.

Treatment

The procedure used in this study was a modification of the method described in "Delayed Contact Hypersensitivity in the Guinea-pig" Buehler, E.V. (1965), Arch. Dermatol. 91, 171.

Induction

Test material
Prior to each induction application, the skin on the left shoulder region of the guinea pig was clipped free of hair.

A 2 x 2 cm patch of surgical gauze (3 layers thick) was saturated with approximately 0.5 ml of test material (50% w/w in distilled water). The patch was placed on the skin and covered by impermeable plastic adhesive tape (5 cm width). This in turn was firmly secured by elastic adhesive bandage (5 cm width) wound round the torso of the animal and fixed with impervious plastic adhesive tape. The exposure period for each induction dose was approximately 6 hours. At the end of each exposure period, the dressings were removed and any resulting dermal reactions were assessed approximately 24 hours later for erythema and edema according to the scale shown on Appended page 1. Nine induction applications were made over a 3 week period.

Controls
The control animals were subjected to the same procedures as the test animals except that the test compound was omitted.

Positive controls
These animals were also subjected to the same procedures as those administered the test material except that the administered material was 25% formalin (v/v in distilled water). The 2 x 2 surgical gauze pad was saturated with approximately 0.5 ml of this solution.

A control group for the positive controls was subjected to the described induction procedure without applications of formalin.

Results

Test material
No dermal reactions were seen in any of the test or control animals (Appended pages 2 and 3).

Positive controls
Well defined to severe dermal reactions were seen in all ten test animals No dermal reactions were seen in the controls (Appended pages 4 and 5).

Challenge

Test material
The test and control animals were challenged topically 2 weeks after the ninth induction application using SAN 619F 40 WDG, 50% w/w in distilled water.

235

Hair was clipped from a 25 cm^2 area on the right flank of each animal. The skin was exposed to the test material by the same procedure described for the induction applications for 6 hours. A second challenge application was made 4 days later in order to verify the results of the first challenge; the method used was similar to that used for the first challenge except application was made on the left flank.

Following the challenge dose, the application site was evaluated 24, 48 and 72 hours after removal of the patch. Reactions were scored according to the scale on Appended page 1.

Dermal reactions in the test animals induced by the challenge applications were compared with the findings simultaneously obtained in the control animals. A dermal reaction which was definitely more marked and/or persistent than the maximum reaction seen in animals of the control group was considered to be positive evidence of delayed contact hypersensitivity. If the dermal reaction in the test animal was only slightly more marked and/or persistent than (but not clearly distinguishable from) the maximum reaction seen in control animals, the result for that test animal was classified as inconclusive. If the dermal reaction was the same as, or less marked and/or persistent than the maximum reaction seen in the control group, the test animal was considered to show no evidence of delayed contact hypersensitivity.

Positive controls
The positive control and formalin control were challenged topically 2 weeks after the ninth induction application using formalin, 15% v/v in distilled water.

Scoring of dermal reactions, application of the second challenge dose of 15% formalin and evaluation of the challenge dermal reactions were performed following the methods described for test material above.

Results

Test material (Appended pages 6 and 7)
No dermal reactions were seen in any of the test or control animals following the first or second challenge application.

Positive controls (Appended pages 8 and 9)
First challenge: one test animal had a well defined erythema, 8 had slight erythema and 1 had no reaction; none of the controls had a dermal reaction.

Second challenge: nine of ten test animals had dermal reaction scores which indicated a positive response. Although well defined erythema was seen in 1 control animal, dermal reaction scores in the remaining control animals were exceeded by the noted test animal scores.

Body weights

Body weights were measured for all animals at the beginning and end of the study.

Results (Appended pages 10 and 11)

Test material
Test and control animals gained 63.5 and 70.3% of respective initial mean body weights.

236

Positive controls
Positive and formalin control animals gained 60.2 and 71.4% of respective initial mean body weights.

CONCLUSION

Repeated topical applications of SAN 619F 40 WDG (50% w/w in distilled water) in guinea pigs did not induce delayed contact hypersensitivity under the conditions of this study.

Core-classification: minimum

237

CYPROCONAZOLE Tox review 007003

Page_____ is not included in this copy.

Pages 238 through 248 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.